Psychological and Biological Sequelae of Exposure to Prenatal Maternal Depression Findings from the 25-Year Prospective South London Child Development Study

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PSYCHOLOGICAL AND BIOLOGICAL SEQUELAE OF EXPOSURE TO PRENATAL MATERNAL DEPRESSION: FINDINGS FROM THE 25-YEAR PROSPECTIVE SOUTH LONDON CHILD DEVELOPMENT STUDY

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Dedicated to the memory of my father (1949-2013) and to my mother

Abstract

Background: A wealth of studies has demonstrated the detrimental effects of exposure to maternal stress *in utero* on emotional psychopathology in childhood. Many of these effects have been attributed to foetal programming of offspring brain development during gestation. Research has also demonstrated an association between exposure to prenatal maternal depression and offspring childhood maltreatment. The main aim of this thesis is to investigate the long-term effects of offspring exposure to prenatal maternal depression on depressive psychopathology in young adulthood, and whether exposure to childhood maltreatment contributes to this association. Secondary aims are to characterise the biological characteristics of young adult offspring who were exposed to prenatal maternal depression, in terms of hypothalamic-pituitary-adrenal (HPA) axis function, inflammation and metabolic function. Previous research has shown that abnormalities in all three systems are associated with exposure to prenatal maternal depression, childhood maltreatment and depressive psychopathology.

Methods: The sample comprised 103 offspring from the South London Child Development Study (SLCDS), a prospective longitudinal birth cohort study setup in 1986 that had followed mothers and their offspring from pregnancy to 16 years. This PhD thesis continued the SLCDS by assessing the offspring at age 25. Data on offspring exposure to depression *in utero* (20 and 36 weeks gestation), childhood maltreatment (birth to 17 years) and young adulthood DSM-IV depressive disorders (18 to 25 years) were obtained through one-to-one clinical interviews. Biological measures of offspring HPA axis function, inflammation and metabolic function were obtained at 25 years.

Results: Offspring exposed to prenatal maternal depression were significantly more likely to have a DSM-IV depressive disorder in young adulthood (18 to 25 years) compared to offspring not so exposed. Offspring exposure to childhood maltreatment and further maternal depression

during childhood were found individually to mediate this association. Offspring exposed to prenatal maternal depression also exhibited significantly greater systemic inflammation at 25 years compared to non-exposed offspring, whilst offspring exposed to childhood maltreatment demonstrated HPA axis abnormalities compared to non-exposed offspring. No effect of early life adversity on metabolic function was observed.

Conclusions: Exposure to prenatal maternal depression results in persistent psychological and biological changes in the offspring that are observable during young adulthood. Childhood maltreatment contributes to these pathologies. These findings have direct implications for policy development and clinical practice: identification and treatment of maternal depression during pregnancy could have a direct impact on reducing levels of child maltreatment as well as depression in the young adult population.

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List of abbreviations

ACEs	Adverse childhood experiences
ACTH	Adrenocorticotrophic hormone
ALSPAC	Avon Longitudinal Study of Parents and Children
AUC	Area under the curve
AUC_G	Area under the curve with respect to ground
AUC_I	Area under the curve with respect to increase
BDI	Beck Depression Inventory
BMI	Body mass index
C	Confounding variable in mediation analyses
CAR	Cortisol awakening response
CECA.Q	Childhood Experience of Care and Abuse Questionnaire
CHD	Coronary heart disease
CHOL	Cholesterol
CI	Confidence interval
CIS	Clinical Interview Schedule
CPA	Child physical abuse
CRH	Corticotrophin-releasing-hormone
CRP	C-reactive protein
CSA	Child sexual abuse
CVD	Cardiovascular disease
DMHDS	Dunedin Multidisciplinary Health and Development Study
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision
EGIR	European Group for the Study of Insulin Resistance
EPDS	Edinburgh Postnatal Depression Scale

FOAD	Foetal origins of adult disease
GC	Glucocorticoid
GCSE	General Certificate of Secondary Education
GP	General practitioner
GR	Glucocorticoid receptor
GRHC	Gallions Reach Health Centre
HAM-D	Hamilton Rating Scale for Depression
HbA1c	Glycosylated haemoglobin
HDL-C	High-density lipoprotein cholesterol
HPA	Hypothalamic-pituitary-adrenal
hsCRP	High sensitivity C-reactive protein
ICD-9	International Classification of Diseases 9th Revision
IDF	International Diabetes Federation
IL	Interleukin
IR	Insulin resistance
KCH	King's College Hospital
KCL	King's College London
LDL-C	Low-density lipoprotein cholesterol
LPS	Lipopolysaccharide
LRGP	Lambeth Road Group Practice
M	Mean
M	Mediator variable in mediation analyses
MDD	Major depressive disorder
mRNA	Messenger RNA
NOS	Not otherwise specified
NSPCC	National Society for Prevention of Cruelty to Children
NVQ	National Vocational Qualification

PBMC	Peripheral blood mononuclear cell
PTSD	Posttraumatic stress disorder
RNA	Ribonucleic acid
SADS	Schedule for Affective Disorders and Schizophrenia
SADS-L	Schedule for Affective Disorders and Schizophrenia, Lifetime Version
SCID-CV	Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version
SD	Standard deviation
SE	Standard error
SES	Socioeconomic status
SLCDS	South London Child Development Study
TG	Triglyceride
TNF-α	Tumor necrosis factor alpha
W	Moderating variable in mediation analyses
WC	Waist circumference
WHO	World Health Organization
X	Antecedent variable in mediation analyses
Y	Consequent variable in mediation analyses

CHAPTER 1: INTRODUCTION

1.1. Overview

Dante Cicchetti, in his influential editorial "The Emergence of Developmental Psychopathology" (1984), articulates a case for the strength and appeal of developmental psychopathology, as a distinct discipline, as resting upon its integrative and explanatory qualities. Indeed, the developmental psychopathologist of the twenty-first century routinely investigates both abnormal and normal human pathology within a developmental framework and at multiple levels of analysis (Cicchetti & Cohen, 2006). Armed with these methodological techniques, psychological and medical theory can be webbed together in an attempt to provide a comprehensive account of human adjustment across the lifespan (Sroufe & Rutter, 1984).

It is with such a perspective in mind, that this thesis aims to investigate foetal programming of depression and neurobiological and metabolic dysfunction in young adulthood. This investigation is operationalized in the context of exploring the impact of foetal exposure to maternal depression during pregnancy on young adulthood depressive disorders and on neuroendocrinological, inflammatory and metabolic function at twenty-five years of age.

The purpose of the introductory background that follows is to provide an overview of the pertinent literature on each of these topics, with a focus on the relationship of these biological systems to depression and early life stress (prenatal maternal stress and child maltreatment). First, a brief overview of depression, foetal programming and child maltreatment is provided. Next, I review the literature linking these three adversities. In the second section, I examine alterations in the three biological systems in the context of depression, and as function of exposure to early life stress. Research is drawn from a mixture of high-quality review articles and contemporary empirical papers.

1.2. Major depressive disorder

1.2.1. Phenomenology

Major depressive disorder (MDD) is a highly prevalent mental disorder characterised by a broad symptomatology, with a multifactorial aetiology. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR; American Psychiatric Association, 2000), a diagnosis of MDD is rated if an individual has suffered from at least one core symptom of (i) depressed mood or (ii) diminished interest or pleasure in activities, and at least three of the following symptoms: (iii) significant weight change or change in appetite; (iv) insomnia or hypersomnia; (v) psychomotor agitation or retardation; (vi) fatigue or loss of energy; (vii) feelings of worthlessness or excessive or inappropriate guilt; (viii) diminished ability to think or concentrate or indecisiveness; (ix) recurrent thoughts of death, recurrent suicidal ideation or plans for committing suicide. These symptoms must be present for most of the day, nearly every day, for at least two weeks. Furthermore, the symptoms must cause significant distress or impairment in normal functioning, and should not be attributable to the death of a loved one, a general medical condition or substance use (American Psychiatric Association, 2000).

MDD is one of the main contributors to the burden of disease worldwide (Kessler, Zhao, Blazer, & Swartz, 1997). It has a lifetime prevalence of 16.5% (Kessler, Berglund, et al., 2005) and a 12-month prevalence of 6.7% (Kessler, Chiu, Demler, & Walters, 2005). Mean age of onset is estimated at 32 years of age, with 18-29 year olds estimated to be 70% more likely to experience lifetime depression than 60+ years olds (Kessler, Berglund, et al., 2005) and for 12-month prevalence depression, they are 200% more likely to experience a depressive episode than are 60+ years olds (Kessler, Chiu, et al., 2005). One of the most consistent sociodemographic correlates of MDD across populations is female gender (Andrade et al., 2003; Kessler, 2003).

1.2.2. Aetiology

MDD has a multifactorial aetiology that is characterised by interplay between environmental and genetic risks. Research has identified numerous psychosocial and biological vulnerability factors that increase the emergence of the disorder, but with no single risk factor being found to be wholly accountable for its emergence. Rather, multiple biopsychosocial pathways have been identified in the pathogenesis of depression. Key biological mechanisms argued to be involved in the pathogenesis of depression include dysregulation in neuroendocrinological systems (Pariante & Lightman, 2008; Pariante, Nesse, Nutt, & Wolpert, 2009), inflammation (Raison, Capuron, & Miller, 2006), dysregulation in neurotransmission (Hirschfeld, 2000; von Wolff, Hölzel, Westphal, Härter, & Kriston, 2013), the genetic transmission of temperament (Sullivan, Neale, & Kendler, 2000), and abnormalities in brain structure (Jacobs, Van Praag, & Gage, 2000) and function (Leppänen, 2006). Key psychosocial theories include the stress/vulnerability model for depression (G. W. Brown & Harris, 1986; G. W. Brown & Prudo, 1981), including the impact of stressful life events (Kendler, Karkowski, & Prescott, 1999) and childhood maltreatment (Bifulco & Moran, 1998; Widom, DuMont, & Czaja, 2007).

1.2.3. Depression during pregnancy

A meta-analysis was conducted in 2004 into the prevalence of prenatal depression (Bennett, Einarson, Taddio, Koren, & Einarson, 2004). The meta-analysis included studies published since 1980. Twenty-one studies were identified, seven of which were based on clinical diagnoses of depression rated through structured clinical interviews (Schedule for Affective Disorders and Schizophrenia; SADS), whilst the remaining studies were based on ratings of depression captured through self-report measures such as the Edinburgh Postnatal Depression Scale (EPDS) and the Beck Depression Inventory (BDI). The authors report the point prevalence of depression by trimester, with rates of 7.4%, 12.8% and 12.0% in the first, second and third trimesters, respectively. Prevalence estimated through structured interviews did not differ significantly

from rates attained through the EPDS, but were significantly lower that rates attained through the BDI.

In a further meta-analysis, Gavin and colleagues (2005), reviewed studies where depression during pregnancy had been rated through only structured clinical interviews. The authors report a period prevalence rate (conception to birth) for MDD of 12.7%, with this rising to 18.4% when ratings of minor depression (akin to the DSM-IV diagnosis of major depressive disorder not otherwise specified [MDD NOS] ; American Psychiatric Association, 2000) were included. In both studies there were noteworthy methodological flaws, such as the exclusion of women with previous psychiatric histories and of low socioeconomic status. Given that both of these factors are well-known risk factors for depression (Gotlib & Hammen, 2009), it is likely that these estimates are conservative, and thus these findings need to be interpreted bearing these caveats in mind.

1.3. Foetal programming

The foetal origins of adult disease (FOAD) hypothesis is the notion that exposure to intrauterine insults during gestation affects foetal brain development, leading to vulnerabilities for a variety of neuropsychiatric and chronic physical health diseases in adult life. This concept was initially termed the "Barker Hypothesis", in acknowledgement of the late David Barker, who, in his seminal 1995 paper presented the argument for an association between poor foetal growth and elevated cardiovascular disease (CVD) in adulthood. Barker and colleagues' research was predominantly drawn from a series of studies on a British cohort of men and women that demonstrated an inverse relationship between low birth weight and death from CVD, and elevated markers of the metabolic syndrome (D Barker, Gluckman, et al., 1993; D Barker, Hales, et al., 1993).

Barker's original hypothesis (D Barker & Clark, 1997; D Barker, 1995) postulates that offspring exposure to adverse conditions during gestation, especially malnutrition, ultimately leads to "programming" of insulin resistance and glucose intolerance. This phenomenon resonates with an evolutionary adaptation, functioning to enhance the foetus' ability to retain as much nutritional energy as possible, resulting in the development of the "thrifty phenotype". It is argued that this physiological reprogramming translates into a risk for metabolic diseases in later life in scenarios where high-energy food becomes readily available. This concept of "mismatch" between the foetal environment and post birth environment in predicting pathological conditions has been replicated in animal studies. It is worth noting that "mismatch" is argued to be a U-shaped phenomenon in which both overabundance and scarcity in gestational environmental conditions can result in the foetus making short-term adaptations that are non-beneficiary in the long run. For example, studies in rodents have shown that maternal high fat diet during pregnancy leads to the greatest obesity in pups fed a normal diet after birth, whilst a maternal low calorie diet leads to obesity in offspring that go on to be fed a high fat diet postnatally (Howie, Sloboda, Kamal, & Vickers, 2009; Thompson et al., 2007).

Since its inception into the wider research field, the Barker Hypothesis has been applied to a variety of offspring *in utero* risk exposures, as well as to a variety of later neuropsychiatric conditions, including emotional and behavioural psychopathology. Specifically, prenatal exposures that cause distress to the mother have been studied. These have included maternal experience of severe stressful life events during the antenatal period, such as suffering bereavement or witnessing a terrorist attack, as well as maternal experience of depression and anxiety. In these latter instances (maternal experience of depression and anxiety during pregnancy), it has been argued that such symptomatology is the consequence of being stressed, and that such psychological distress experienced by the mother may be a risk factor for her developing foetus. The term "prenatal stress" has become the widest and most generic term used to characterise such maternal experiences of distress during pregnancy (Glover, 2011). In detailing the available literature, I use the term "prenatal maternal stress" to conceptualise maternal experience of distress during pregnancy, including both severe stressful life events and depression and anxiety.

This keen interest and widespread application of foetal programming models to a multitude of prenatal risks and pathological conditions, led to the expansion of the term from the Barker Hypothesis to the FOAD hypothesis. Accordingly, research in the last decade has primarily focused on identifying biological mechanisms responsible for the translation of *in utero* adversity into vulnerability for adulthood disease (Charil, Laplante, Vaillancourt, & King, 2010; Reynolds, 2013; Seckl & Holmes, 2007), with a large body of research focusing specifically on the effects of prenatal maternal stress (Glover, 2011; Huizink, Mulder, & Buitelaar, 2004; Van Den Bergh, Mulder, Mennes, & Glover, 2005). The literature presented in this chapter will centre around the effects of prenatal maternal stress, in particular mood disturbances, on offspring psychopathology and reactivity in neuroendocrine, inflammatory and metabolic systems. This PhD thesis also refers directly to this model, as it will specifically look at the impact of exposure

to prenatal maternal depression on depressive psychopathology in young adulthood, as well as dysfunction in neuroendocrine, inflammatory and metabolic systems.

1.4. Prenatal maternal stress and offspring psychopathology

Over the past two decades a wealth of research has been conducted into the effects of offspring exposure to prenatal maternal stress on emotional and behavioural adjustment. The earliest observations for this link come from retrospective cohort studies that involved mothers who were pregnant during periods of natural adversities. For example, Brown and colleagues (2000) reported that offspring of mothers who experienced famine during the Dutch Hunger Winter of 1944-1945, showed elevated rates of MDD in their own adult lives in comparison with offspring of mothers who were pregnant during the same years but did not experience famine. In another study, Meijer (1985) found that children of mothers who were pregnant during the Israeli Six-Day War in 1967 showed behavioural problems in comparison with offspring of mothers who were pregnant in 1969. Whilst these findings suggest an association between maternal emotional state during pregnancy and offspring affective and behavioural psychopathology, they are limited by methodological weaknesses that are an artefact of their retrospective nature. A major caveat is the inability to control for unmeasured confounders, such as other maternal characteristics that could account for the observed associations (Thapar & Rutter, 2009). Thus, one cannot be confident from retrospective studies alone that these links are not in fact spurious.

In contrast, prospective longitudinal studies, many of which have been setup in the past couple of decades, are better able to control for the influence of potential confounders, thus offering more robust tests of foetal programming effects by maternal experience of stress during pregnancy. Indeed, the prospective design is one of the core strengths of this thesis. Prospective studies have demonstrated an association between numerous types of maternal prenatal stress, such as daily hassles, life events and mood disturbances, on a variety of offspring neurodevelopmental outcomes, including behavioural, emotional and cognitive problems (Glover, 2011; Huizink et al., 2004; Talge, Neal, & Glover, 2007; Van Den Bergh et al., 2005). A

common feature of these prospective studies is their exploration of offspring outcomes during childhood (infancy into adolescence).

In a study of 22 mother-infant dyads, maternal prenatal anxiety and depression were significantly associated with infant negative behavioural reactivity to novelty (Davis et al., 2004). No associations were observed for maternal postnatal depression and anxiety. In another study of 170 mothers and their 8 month old infants, high levels of pregnancy-specific anxiety was found to predict lower mental and motor developmental in the offspring (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003). Additionally, high amounts of self-reported daily hassles during pregnancy were also associated with lower mental developmental scores at 8 months. In a study of 71 Dutch mothers and their eight year old children, Van den Bergh and colleagues (2004) found that maternal prenatal anxiety during pregnancy explained, respectively, 15%, and 9% of the variance in cross-situational offspring externalizing problems, and self-report anxiety, even after controlling for child's gender, parents' educational level, birth weight, and postnatal maternal anxiety. At 15 years, maternal prenatal anxiety was found to predict depressive symptomology in the offspring (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). Similarly, all effects remained after controlling for maternal prenatal smoking, birth weight, obstetrical outcomes, maternal postnatal anxiety and offspring puberty development.

Further strong evidence for an association between maternal prenatal mood and offspring outcome comes from the Avon Longitudinal Study of Parents and Children (ALSPAC). The ALSPAC is a unique British birth cohort comprising detailed prospectively collected information on over 14,000 mothers and their offspring. In a study including over 7,000 mother-child dyads, O'Connor and colleagues (O'Connor, Heron, et al., 2002; O'Connor, Heron, Golding, et al., 2002) showed that offspring of mothers who were prenatally anxious were twice as likely to exhibit behavioural depressive and anxious psychopathology at four years, compared to offspring of

non-prenatally anxious mothers These effects persisted into middle childhood (O'Connor, Heron, Golding, & Glover, 2003). Furthermore, when the offspring reached adolescence (13 years) maternal prenatal anxiety continued to predict disruptive behaviour symptomatology (E Barker & Maughan, 2009; O'Donnell, 2010) and emotional symptomatology (O'Donnell, 2010). Importantly, the aforementioned effects of maternal prenatal anxiety were found to be independent to the effects of prenatal smoking and drinking, obstetric outcomes, psychosocial disadvantage, maternal age and maternal postnatal anxiety and depression.

Turning more specifically to the influence of prenatal maternal depression, neonates born to mothers who were depressed during pregnancy have been shown to be less reactive to auditory stimulation in comparison to neonates born to non-depressed mothers. In our South London Child Development Study (SLCDS), a prospective longitudinal birth cohort study of over 150 mother-offspring pairs, offspring of mothers who suffered clinical depression during pregnancy were found to have greater depression and conduct problems in adolescence compared to offspring of mothers who were not clinically depressed in pregnancy (Hay, Pawlby, Waters, Perra, & Sharp, 2010; Hay, Pawlby, Waters, & Sharp, 2008; Pawlby, Hay, Sharp, Waters, & O'Keane, 2009; Waters, 2008). Notably, prenatal maternal depression significantly predicted offspring psychopathology after controlling for the family environment, the mother's smoking and drinking during pregnancy, and parents' antisocial behaviour. The effects on offspring conduct disorder were independent of maternal postnatal depression, whilst the effects on offspring depression were partially mediated by maternal postnatal depression. Findings from the ALSPAC have also provided evidence for the influence of prenatal maternal depression. O'Donnell (2010) showed that in over 9,000 mother-offspring pairs, prenatal maternal depression was associated with offspring emotional and behavioural problems at 13 years, independent of the effects of prenatal maternal anxiety and other potential confounders.

Finally, findings from animal studies of the effects of prenatal maternal stress on the offspring are comparable to those found in the human study literature. For example, studies in rodents show that pups of prenatally stressed dams showed greater depressive-like behaviour, such as decreased exploratory behaviour and reduced feeding behaviour, compared to pups of non-stressed dams (Weinstock, 2008).

Collectively, these findings provide robust evidence for an association between prenatal maternal stress and offspring psychopathology. Moreover, there is keen evidence that prenatal maternal depression specifically, is associated with detrimental offspring emotional and behavioural problems in childhood and adolescence. The fact that wide varieties of stressors in pregnancy are shown to affect a wide variety of psychopathologies in the offspring, suggests that prenatal maternal stress generates general, non-specific neurobehavioural vulnerability in the offspring, that has the potential to manifest in an array of emotional, behavioural and developmental problems. This observation suggests an important point. It advocates the notion that the hormonal environment afforded by the mother may programme underlying neurobiological systems that are component facets of emotional and behavioural development. Further studies are needed to investigate the persistence of these effects into adulthood, with particular attention to the development of depression. Levels of depression tend to increase through the transition period from childhood, adolescence and young adulthood (Costello, Copeland, & Angold, 2011). Also, depression in adolescence is commonly preceded by disruptive behavioural disorders (Hipwell et al., 2011). These data would suggest that programming effects may be particularly pertinent to the manifestation of depressive disorders in adulthood.

1.5. Child maltreatment

Child maltreatment is a major public health issue. The National Society for Prevention of Cruelty to Children (NSPCC) identifies child maltreatment as any exposure to physical abuse, sexual abuse, emotional abuse, neglect or domestic violence. In the wider research literature, the terms "adverse childhood experiences" (ACEs), "victimization" and "trauma" have been applied in a more generic manner to refer to the aforementioned forms of abuse and neglect, as well as to other negative childhood experiences such as peer bullying, exposure to major traumatic life events and parental loss (Preer, Sorrentino, Ryznar, & Newton, 2013). However, the most widely accepted definition of child maltreatment comprises child sexual abuse (CSA), child physical abuse (CPA), child emotional abuse (sometimes referred to as psychological abuse) and child neglect. Table 1 provides definitions for these forms of abuse and neglect (Norman et al., 2012).

Currently, over 40,000 children in England are on the child protection register (Department for Education, 2012). Over 18,000 children are registered for being exposed to neglect, over 4,000 are registered for being exposed to CPA, over 2,000 are registered for exposure to CSA and over 12,000 are registered for exposure to emotional abuse. Global prevalence rates for child maltreatment remain uncertain, with estimates varying between 2% and 67% (Norman et al., 2012). These discrepancies are likely because much violence against children remains hidden and unreported due to fear and stigma, as well as due to a general societal acceptance of this type of violence.

Table 1. Common definitions of child maltreatment

Type of maltreatment	Description
Child physical abuse	Physical abuse of a child is defined as the intentional use of physical force against a child that results in, or has a high likelihood of resulting in, harm for the child's health, survival, development, or dignity. This includes hitting, beating, kicking, shaking, biting, strangling, scalding, burning, poisoning, and suffocating. Much physical violence against children in the home is inflicted with the object of punishing.
Child sexual abuse	Sexual abuse is defined as the involvement of a child in sexual activity that he or she does not fully comprehend, is unable to give informed consent to, or for which the child is not developmentally prepared or else that violates the laws or social taboos of society. Sexual abuse can be perpetrated by adults and other children who are, by virtue of their age or stage of development, in a position of responsibility, trust, or power over the victim.
Child emotional abuse	Emotional and psychological abuse involves both isolated incidents, as well as a pattern of failure over time on the part of a parent or caregiver to provide a developmentally appropriate and supportive environment. Acts in this category may have a high probability of damaging the child's physical or mental health, or his/her physical, mental, spiritual, moral, or social development. Abuse of this type includes the following: the restriction of movement; patterns of belittling; blaming; threatening; frightening; discriminating against, or ridiculing; antipathy; and other non-physical forms of rejection or hostile treatment.
Child neglect	Neglect includes both isolated incidents, as well as a pattern of failure over time on the part of a parent or other family member to provide for the development and well-being of the child, where the parent is in a position to do so, in one or more of the following areas: health; education; emotional development; nutrition; shelter; safe living conditions. The parents of neglected children are not necessarily poor.

Note. Adapted from Norman et al., 2012.

1.6. Child maltreatment and affective disorders

Child maltreatment has repeatedly been shown to predict a variety of psychiatric disorders in adulthood, including mood disorders, substance use disorders and psychotic disorders (Green et al., 2010; Keyes et al., 2012; Norman et al., 2012). Furthermore, in regards to risk specifically for depression, studies have shown that a range of abuse types (e.g. sexual, physical, emotional) and forms of neglect (e.g. physical, emotional) are associated with elevated risk for depression (Antonia Bifulco, Moran, Baines, Bunn, & Stanford, 2002; Fergusson, Boden, & Horwood, 2008; Green et al., 2010; Keyes et al., 2012; Lieberman, Chu, Van Horn, & Harris, 2011; Nanni, Uher, & Danese, 2012; Nemeroff, 2004; Norman et al., 2012; Widom et al., 2007). Some studies have suggested a 'dose-response' relationship between severity of maltreatment and depression (Antonia Bifulco et al., 2002; Wise, Zierler, Krieger, & Harlow, 2001), whilst a recent meta-analysis revealed an association with unfavourable (i.e. recurrent or chronic) courses of MDD (Nanni et al., 2012). Preclinical studies in rodents and non-human primates have also shown that prolonged separation during the early sensitive period of development leads to behavioural changes in the offspring, that persist into adult life, and resemble depressive and anxious human symptomatology (Nemeroff, 2004).

These cumulative findings also support the notion that childhood maltreatment may exert its effects through generalised risk vulnerabilities for psychopathology, such as dysfunction in neuroregulatory mechanisms, rather than through individual pathways to specific disorders. Indeed, Keyes and colleagues (2012) conducted a specific test of this hypothesis. They demonstrated in a nationally representative sample of 34,653 American adults, that the effects of child abuse (physical, sexual, emotional abuse) on affective and substance use disorders were fully mediated by dysregulation in latent generalised internalising and externalising dimensions, rather than specific disorders.

1.7. Prenatal maternal stress and offspring child maltreatment

1.7.1. Predictors of child maltreatment

It has been observed that child maltreatment potential is highest amongst families that experience cumulative environmental adversities (Begle, Dumas, & Hanson, 2010). Key risk markers for child maltreatment potential include parental mental health problems, parental antisocial traits, low socioeconomic status (SES), parental experience of maltreatment in their own childhood, parental financial, housing and relationship difficulties, and offspring disruptive behaviour problems (Appleyard, Berlin, Rosanbalm, & Dodge, 2011; Berlin, Appleyard, & Dodge, 2011; Jaffee et al., 2004; Lereya & Wolke, 2013; Plant, Barker, Waters, Pawlby, & Pariante, 2013; Sidebotham & Golding, 2001; Sidebotham & Heron, 2003, 2006; Thornberry, Knight, & Lovegrove, 2012). These findings suggest that risk for child maltreatment potential can be broadly separated into two non-mutually exclusive mechanisms: (i) child-driven effects and (ii) parent-driven effects.

1.7.2. Prenatal maternal stress as a risk factor for offspring child maltreatment

In accordance with a child-driven effects model, the literature reviewed in section 1.4 that demonstrates a robust link between maternal prenatal stress and offspring disruptive behaviour, supports the idea that prenatal maternal stress is a key risk factor for child maltreatment potential. That is, the argument follows that prenatal maternal stress can indirectly increase the risk of child maltreatment potential through increasing the child's propensity for elevated disruptive behaviour, which in turn may elicit detrimental parenting practices. Indeed, in an adoption study of adolescents and their adoptive and biological parents, Ge and colleagues (1996) found (i) that adolescents' hostile and antisocial behaviour was correlated with their biological parents' antisocial behaviour, and (ii) adoptive parents of adolescents who showed disruptive behaviours exhibited harsher parenting practices which were positively correlated

with the child's biological parents' behavioural problems. This data provides support for offspring-elicited harsher parenting as a function of the offspring's disruptive characteristics. However, a limitation to this premise is that it may not be as applicable to neglect, as it is to abuse. Also, parents are not always the perpetrators of child abuse.

A parent-driven model can be argued to have greater power in accounting for risk for child maltreatment. Whilst research has illustrated that maternal experience of mental health difficulties increases the risk of offspring childhood maltreatment (see 1.7.1), less research has focused on whether maternal mood disturbances specifically during pregnancy are associated with greater levels of childhood maltreatment. A meta-analysis was conducted in 2008 into the relationship between maternal mental health problems and maternal-foetal attachment (Alhusen, 2008). Overall findings, from a review of 22 studies, revealed that maternal depression and anxiety during pregnancy was associated with lower maternal-foetal attachment. Furthermore, rates of child-mother secure attachment were shown to be lower amongst maltreated preschool children, whilst rates of disorganized attachment were found to be higher, in comparison with non-maltreated children (Stronach et al., 2011). Together, these data give support to the notion that mothers who suffer depression and anxiety during pregnancy may go on to have poorer parent-child relationships with their offspring, putting the offspring at risk for greater exposure to neglect and abuse.

In support of this premise, data from our SLCDS revealed that offspring of mothers who experienced prenatal depression were 4 times more likely to experience child maltreatment between birth and age 11, in comparison with offspring of mothers who were not depressed during pregnancy (Pawlby, Hay, Sharp, Waters, & Pariante, 2011). This association was independent of the effects of maternal postnatal depression and other prenatal risks. Notably, in all instances of CSA, the perpetrator was not a parent. These data suggest several important points. First, they provide a clear association between maternal depression specifically during

the antenatal period and offspring exposure to maltreatment. Second, they illustrate that it is not necessarily the case that mothers who are depressed in pregnancy go on to abuse their offspring; rather, such mothers appear less able to protect their young, indirectly exposing them to elevated risks of abuse.

Moreover, in a recent study conducted using data from the ALSPAC (*n* = 8,829), Lereya and Wolke (2013) found that prenatal maternal depression and anxiety predicted offspring peer victimisation at age 8. This effect was observed after controlling for gender, partner conflict, offspring temperament, postnatal family adversity and other stressors during pregnancy. In tests for indirect effects, prenatal maternal depression and anxiety were found to lead indirectly to peer victimisation through maladaptive parenting styles (hitting, shouting, parent hostility etc.). These data complement the findings from our South London Child Development Study, and provide novel information on the potential mechanisms (parental-driven effects) of the association between prenatal maternal affective psychopathology and offspring experiences of abuse and neglect.

Animal models have also been used to test whether maternal stress during pregnancy alters maternal behaviour after birth. One of the best models in which this has been examined is through assessing licking and grooming (LG) behaviour in rodents, as an index of maternal care. Champagne and colleagues (2006) have conducted some of the clearest investigations into this phenomenon. In a series of studies, they demonstrated that dams that naturally expressed high LG under normal circumstances, exhibited low LG at six days post-birth after being subjected to seven days of restraint stress during pregnancy. Furthermore, dams that were subjected to prenatal stress also exhibited decreased oxytocin levels in the postnatal period. Higher human maternal oxytocin levels during pregnancy have been found to be positively associated with maternal bonding behaviour, attachment-related thoughts and frequency of checking of the

infant (Feldman, Weller, Zagoory-Sharon, & Levine, 2007), as well as protective of the occurrence of postnatal depression (Skrundz, Bolten, Nast, Hellhammer, & Meinlschmidt, 2011).

There is also evidence supporting the notion that environmental experiences after birth can modulate the effects of prenatal maternal stress. In our South London Child Development Study, we found that offspring exposure to child maltreatment increased the risk of adolescent depression by four fold (Pawlby et al., 2011). In a sample (*n* = 271) of mother-child pairs drawn from the Wirral Child Health and Development Study, prenatal maternal depression was found to predict increased infant (10 months) negative emotionality and poorer physiological adaptability (measured via response to vagal withdrawal from a stressor) in only offspring whose mothers showed low stroking behaviour (H Sharp et al., 2012). These studies suggest that adversity during foetal life and after birth affect similar underlying physiological and psychological domains.

These research findings show prenatal maternal stress to be a distinct risk factor for child maltreatment potential. Mechanisms appear to be indirect through two non-mutually exclusive pathways: (i) elevation of offspring disruptive behaviour which may in turn elicit harsher parenting practices (child-driven effects); (ii) down-regulation of maternal care, thereby increasing neglectful behaviour and increasing risk of exposure to abuse (mother-driven effects).

1.8. Interim summary

From the research reviewed in this section, we have seen that offspring exposure to maternal stress during gestation can have long lasting detrimental effects on behavioural and emotional adjustment across the childhood years. Much of this impairment manifests as stress-related psychopathology such as depression. Furthermore, gross environmental adversity during childhood, specifically experience of child abuse and neglect (collectively termed child maltreatment), is also strongly associated with affective psychopathology, which manifests predominantly during adulthood. The effects of exposure to early life stress (prenatal maternal stress and child maltreatment) are argued to be mediated by generalised vulnerability to stress reactivity. This notion suggests that dysregulation in biological stress systems may be one putative mechanism for the biological embedding of adversity into vulnerability for stress-related disorders such as depression. Furthermore, the similarity in psychological outcomes between individuals exposed to gross adversity during childhood (i.e. child maltreatment) and during gestation (i.e. intrauterine exposure to maternal stress during pregnancy) resonates equifinality, further alluding to a shared underlying mechanism for the translation of psychosocial adversity into risk for affective psychopathology.

The initial literature on foetal programming effects, as indexed by poor obstetric outcomes, demonstrated negative consequences on cardio-metabolic parameters. These findings support the notion of programming of biological systems relevant to chronic health conditions. Thus, the next section will review the literature on the effects of early life stress on neuroendocrine, inflammatory and metabolic systems. It will conceptualise these systems both as outcomes per se and as potential mediators for the biological embedding of adversity on risk for depression.

1.9. Biological reactivity

The hypothalamus-pituitary-adrenal (HPA) axis, the inflammatory response, and abnormalities in metabolic function have all been found to be influenced by early life stress. In addition each of these systems has been associated independently with depression. This section will explore the link between abnormalities in each of these systems and depression, as well as the impact of early life stress upon them. Thus, they will be treated both as outcomes in their own right, as well as potential mechanisms for the pathogenesis of depression within the context of the influence of early life stress.

1.9.1. HPA axis

A primary function of the HPA axis is modulating the response to stress, which comprises an individual's reaction to external and internal (psychological) stimuli and challenge. HPA axis activity begins with stimulatory input to the hypothalamus, which stimulates secretion of corticotropin-releasing-hormone (CRH) and vasopressin. CRH and vasopressin stimulate the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary into the blood stream, which thereby stimulates the secretion of glucocorticoids (GCs; cortisol in humans, corticosterone in rodents) from the adrenal cortex. Once released, GCs exert their action primarily by binding to glucocorticoid receptors (GRs) found in many body tissues. GCs exert inhibitory effects on the HPA axis through activation of GRs in the hypothalamus, thereby executing negative feedback as a self-regulatory mechanism. The HPA axis is also involved in the modulation of a range of other physiological processes such as metabolism and immunity, as well as psychological processes such as memory.

1.9.1.1. Diurnal rhythm

Cortisol production follows a diurnal rhythm, with levels decreasing through the afternoon, reaching a nadir late at night. Levels then increase towards the end of the sleep cycle and peak around 30 minutes after awakening (Pruessner et al., 1997).

1.9.1.2. Cortisol awakening response

The cortisol awakening response (CAR) is the observation that cortisol levels start to increase after awakening, reaching a peak at around 30 minutes post awakening and tailing off thereafter. Pruessner and colleagues (1997) were amongst the first researchers to characterise the CAR, demonstrating moderate intra-individual reliability across days. The CAR has since become considered a reliable marker of HPA axis activity that appears to be independent to the diurnal profile (Clow, Thorn, Evans, & Hucklebridge, 2004). However, the precise function of the CAR is not clear (Fries, Dettenborn, & Kirschbaum, 2009). Initial theories posited that its purpose was to promote physiological processes, such as metabolic activity, upon awakening (Pruessner et al., 1997). However, studies have since demonstrated awakening cortisol levels to be unrelated to awakening blood glucose levels (Hucklebridge, Clow, Abeyguneratne, Huezo-Diaz, & Evans, 1999). As awakening is akin to a small stressor, the CAR has been interpreted as an index of stress reactivity (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). Indeed, in a systematic review of 147 studies, a higher CAR, with respect to a greater increase in cortisol since awakening, was found to be positively associated with work and general life stress, and negatively correlated with fatigue, burnout and exhaustion (Gonzalez, Jenkins, Steiner, & Fleming, 2009). These findings suggest that the CAR is an index of HPA axis function and stress reactivity.

1.9.2. HPA axis in depression

A common biological characteristic of depression is dysregulation in the HPA axis (Holsboer, 2000). Hyperactivity of the HPA axis has been observed in many studies of depressed individuals, who show increased levels of plasma, salivary and urinary cortisol, as well as larger pituitary and adrenal glands (Nemeroff & Vale, 2005). Impaired function of the GR is thought to result in impaired GC-mediated negative feedback, which it is argued underlies the dysfunction in the HPA axis that is commonly observed in depressed patents. This concept of impairment in GR signalling as a key molecular mechanism of HPA axis dysregulation has been referred to as "GC resistance" (Pariante & Lightman, 2008). Support for the notion of GC resistance in depressed patients comes from a multitude of studies demonstrating that the synthetic administration of the GC dexamethasone, does not lead to suppression of the HPA axis, as measured by GC output, whilst GC suppression is observed amongst healthy individuals (Pariante & Lightman, 2008). Support also comes from animal studies using transgenic mouse strains that under- or overexpress GRs. Such studies have demonstrated that mice with decreased GR expression exhibit greater depressive-like behaviours (Chourbaji, Vogt, & Gass, 2008). Furthermore, *in vitro* studies have consistently shown that antidepressants modulate GR function (Anacker, Zunszain, Carvalho, & Pariante, 2011), further suggesting that the receptor may play a pivotal role in neurobiological disturbances that contribute to depressive psychopathology.

1.9.2.1. Diurnal cortisol and CAR in depression

Whilst it is clearly evident that dysregulation of the HPA axis is a key feature of depression, the exact nature of this dysfunction is not fully understood. Studies investigating atypical diurnal HPA patterns in depressed individuals have produced mixed results, with a wealth of studies reporting hypercortisolemia (Bhagwagar, Hafizi, & Cowen, 2003, 2005; Vreeburg et al., 2009), but others reporting hypoactivity (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013).

For example, in a study of 20 non-medicated depressed patients and 40 healthy controls, depressed patients were found to secrete approximately 25% more cortisol than controls at 30 minutes post-awakening, but had similar cortisol levels 60 minutes after waking (Bhagwagar et al., 2005). In another study of 1,588 Dutch adults, depressed and remitted individuals showed an elevated CAR compared to healthy controls. Evening cortisol levels were also found to be higher amongst depressed individuals at 10pm, but not 11pm, in comparison with controls (Vreeburg et al., 2009). In contrast, Jarcho and colleagues (2013) found in sample of 23 depressed women and 26 matched controls, that depressed women exhibited lower cortisol at 30 minutes post awaking, as well as in the evening, in comparison with controls. Depressed patients also showed less suppression of cortisol following dexamethasone administration than non-depressed women, suggesting GC resistance in these women. One potential explanation for these seemingly paradoxical findings could be that GC output may not be a linear correlate to all functional activity of the HPA axis. That is, whilst impairment in GR-mediated feedback may indeed be present, this could manifest differently depending on the chronicity and severity of disturbance, with a blunted CAR perhaps being indicative of fatigue and exhaustion. These discrepancies could also be explained by methodological differences such as sampling, type of depression, co-morbid conditions, age and early life experiences. What is clear, however, from all of these studies, is that dysfunction in GC secretion is a key facet of depression.

1.9.3. HPA axis and early life stress

Much research has also focused on investigating alterations in the HPA axis in relation to early life stress. HPA axis dysfunction is both a detrimental outcome in its own right, as well as a potential mechanism for the transmission of disease pathology. That is, given the strong association between early life adversities, such as child maltreatment and exposure to depression *in utero* and risk for depression (see 1.4 and 1.6), and the posited involvement of HPA axis dysfunction in the pathogenesis of depression, much investigation has been motivated by the proposition that dysregulation in the HPA axis system may be a key biological mechanism for the translation of psychosocial adversities into biological vulnerabilities for later psychopathology. This section will review the pertinent literature on changes in HPA axis function as a consequence of (i) exposure to depression *in utero* and (ii) exposure to childhood maltreatment, which is the focus of this thesis.

1.9.3.1. Prenatal maternal stress and programming of the offspring HPA axis

Pathophysiological explanations for the reliable association between foetal exposure to prenatal maternal stress and later pathological conditions (see 1.3 and 1.4) have focused primarily on (i) foetal malnutrition and (ii) programming of the HPA axis through excessive exposure to GCs (Seckl & Holmes, 2007). This section will focus on the latter theme.

Much of the initial research into foetal programming of the HPA axis was conducted in animals, yet in the past decade, several studies have involved humans. Findings from early animal studies (primarily rodents and nonhuman primates) revealed that offspring of mothers who were injected with artificial GCs (i.e. dexamethasone, betamethasone) show hyperactivity of the HPA axis, in addition to depressive- and anxiety-like behaviour (Charil et al., 2010; Glover, O'Connor, & O'Donnell, 2010; Reynolds, 2013; Seckl & Holmes, 2007; Weinstock, 2008).

Human evidence for foetal programming of the HPA axis comes from a mixture of studies, some of which have investigated offspring HPA axis function in relation to maternal reports of psychological distress during pregnancy, whilst others have included measurements of maternal cortisol levels during pregnancy in addition to reports of psychological report of stress. In a prospective Dutch sample of 15 year old offspring and their mothers (*n* = 58), prenatal maternal anxiety predicted a lower diurnal cortisol profile, characterised by lower awakening cortisol but higher evening cortisol, in offspring of mothers who were prenatally anxious during pregnancy and in comparison to offspring of non-prenatally anxious mothers (Van den Bergh et al., 2008). Notably, these effects remained after controlling for maternal smoking, birth weight, obstetric outcomes, postnatal anxiety and puberty phase. Furthermore, using data from the ALSPAC, O'Donnell and colleagues (2013) also showed that in a sample of 889 15 year-olds, both maternal prenatal depression and anxiety predicted a blunted CAR (at 30 minutes post-awakening), and an overall flatter diurnal profile, indexed by lower awakening but higher evening cortisol levels. These effects were also independent of the effects of postnatal psychosocial factors.

In terms of studies which directly measured maternal cortisol levels during pregnancy, Davis and colleagues (2011) found that in a sample of 116 mother-infant dyads, higher maternal prenatal cortisol levels positively predicted a larger cortisol response to the heel-stick procedure in their 1 day old infants. Furthermore, poorer infant behavioural recovery from the procedure was observed amongst infants whose mothers reported high distress levels and who exhibited elevated cortisol during pregnancy. In a recent prospective study involving 125 mother-infant pairs, higher levels of amniotic cortisol predicted increased cortisol response to separation-reunion stress at 17 months; infants who were exposed to higher levels of amniotic cortisol showed higher baseline cortisol levels and a blunted cortisol response to the stressor (O'Connor, Bergman, Sarkar, & Glover, 2013). This effect was independent of prenatal, obstetric, SES and attachment factors.

These studies support the premise that foetal exposure to maternal prenatal stress can lead to persistent changes in the HPA axis that are observable up to 15 years later. However, in all of these studies, GC output has been the main measure of HPA axis function. To characterise fully these alterations, examination of further dimensions of the HPA axis are required. Animal studies have demonstrated that excessive prenatal GCs can down-regulate GR density, as indexed by reduced messenger RNA (mRNA) expression in the hypothalamic paraventricular nucleus, with the implication of programming GC resistance in the offspring after birth, and thus stress reactivity (Welberg, Seckl, & Holmes, 2000). In a human study (*n* = 82), Oberlander and colleagues (2008) showed that 3-month-old offspring whose mothers had been depressed during pregnancy exhibited increased methylation at the 1F promoter region of the GR gene, NR3C1, as found in peripheral blood mononuclear cells (PBMCs), in comparison with offspring of non-prenatally depressed mothers. Increased methylation status was further positively associated with elevated offspring cortisol output in response to a stress challenge. In a more recent study, prenatal exposure to maternal stress (severe intimate partner violence) also predicted increased methylation at the 1F region of NR3C1 in their offspring at 10 years of age (Radtke et al., 2011). Although gene expression or DNA methylation status will not be examined in this PhD thesis, DNA and mRNA samples will be collected for biobanking for future analysis.

Finally, research has demonstrated that women's HPA axes are hyperactive during pregnancy, as indexed by elevated circulating cortisol levels (Jung et al., 2011) and increased secretion of placental CRH (Petraglia et al., 1993). Notably, HPA axis activity regulates parturition, with greater activation associated with shorter gestation terms, thus the use of gestational birth weight and gestational age as an indirect measure of an adverse intrauterine environment (Duthie & Reynolds, 2013). In a recent study (*n* = 65), pregnant depressed women were found to have higher CRH concentrations at the second trimester point, and had higher mean evening salivary cortisol concentrations in comparison to healthy controls (O'Keane et al., 2011). Depressed women also had a shorter pregnancy length. Furthermore, Katz and colleagues

(2012) demonstrated that GR functional sensitivity, assessed in PBMCs from a group of 29 pregnant women, was negatively correlated with depressive symptomatology.

Overall, these studies point to a the putative role of foetal exposure to excessive maternal GCs, brought about through excessive distress and mood disturbances during pregnancy, in programming alterations to the offspring's HPA axis. Albeit that many of these studies have notable methodological limitations, such as small sample sizes and potential confounding influence from co-occurring psychosocial and medical factors, yet, collective evidence supports current programming theory and warrants continued investigation into the role of the HPA axis as a putative biological mechanism for the intergenerational transmission of stress reactivity.

1.9.3.2. Child maltreatment and alterations to the HPA axis

A multitude of studies have also characterised childhood maltreatment though its neurobiological sequelae in terms of alterations to the HPA axis (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Nemeroff, 2004). There is a high degree of similarity between the effects of offspring exposure to intrauterine-environmental stress (i.e. prenatal maternal stress) and adverse experiences during childhood, such as exposure to child maltreatment, alluding to the notion that these insults differ primarily by timing, rather than by the nature of their effects on the HPA axis (Gluckman, Hanson, Spencer, & Bateson, 2005).

Indeed, rodent studies have demonstrated that maternal separation results in persistent alterations to the HPA axis (hyperactivity and attenuation), indexed through increased GC response to stressors, increased plasma ACTH, and decreased GR densities in the hippocampus (de Kloet & Oitzl, 2003; Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Sánchez, Ladd, & Plotsky, 2001). Moreover, pups of dams exhibiting low LG behaviour showed decreased GR expression accompanied by increased methylation of the promoter region of NR3C1, suggesting

epigenetic modulation (Weaver et al., 2004). In contrast, enhanced maternal care, as expressed through greater LG behaviour, results in a reduced pup GC response to acute stress, increased hippocampal GR mRNA expression, decreased hypothalamic CRH mRNA and enhanced GC feedback sensitivity (D Liu et al., 1997).

In studies of humans, findings have been more mixed. For example, De Bellis and colleagues (1994) reported a blunted ACTH response to CRH challenge in sexually abused girls in comparison with controls. In contrast, Hart and colleagues (1996) found cortisol hypersecretion in response to stress challenge in a group of maltreated preschool children. Other studies have found alterations in HPA axis parameters to be modulated by the presence of psychopathology (McCrory, De Brito, & Viding, 2010), whereby some studies have reported hypoactivity in the context of maltreated and depressed children (Tarullo & Gunnar, 2006).

Amongst adults, Heim and colleagues (2002) found that in a sample of 49 women, a history of childhood maltreatment was associated with an increased ACTH and response to psychosocial challenge, whilst adulthood life events further amplified these effects. Further studies have observed hypoactivity in maltreated individuals in the context of posttraumatic stress disorder (PTSD). Inconsistencies across these studies may reflect differences in the severity, timing and chronicity of maltreatment, as well as the influence of confounds including the frequently observed comorbidity of depression, PTSD and trauma. In a unique study, McGowan and colleagues (2009) observed increased methylation of GR NR3C1 promoter region and decreased GR mRNA in suicide victims with a history of childhood abuse compared to non-abused suicide victims.

1.9.4. Inflammation

Inflammation is the process of activation of the immune system in the interest of self-preservation. The inflammatory response can occur over a short period of time (acute) or can persist with long course (chronic). Key aspects involved in the inflammatory response are the production of cytokines (e.g. interleukin- [IL] 1, 6, 4, 10, tumor necrosis factor-alpha [TNF-α]) acute phase proteins (e.g. C-reactive protein [CRP]) chemokines and adhesion molecules (Maes, 1995; G. E. Miller, Stetler, Carney, Freedland, & Banks, 2002; Musselman, Miller, et al., 2001). Activation of the immune response leads to the production of cytokines, pro- and anti-inflammatory, which in turn influence pathophysiological domains such as neuroendocrine function, neurotransmitter metabolism and regional brain activity (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Schiepers, Wichers, & Maes, 2005).

1.9.5. Inflammation and the HPA axis

Inflammation and the HPA axis have bidirectional modulatory effects. Under normal circumstances, activation of the HPA axis has potent inhibitory effects on inflammation. However, in cases of dysfunctional HPA axis activity, it has been argued that this usual inhibitory control is no longer effected, thus enabling the activity of inflammatory pathways (G Miller et al., 2008). Inflammation is believed to modulate the neuroendocrine system through two broad mechanisms: (i) activation of the HPA axis and (ii) through direct effects on the GR (Horowitz, Zunszain, Anacker, Musaelyan, & Pariante, 2013). Evidence for the activating effects of cytokines on the HPA axis comes from the paradigm of therapeutic chronic administration of proinflammatory cytokine interferon alpha (IFN-α), which is routinely used for medical illness such as hepatitis C. IFN-α has been observed to active the HPA axis, indexed through elevated ACTH and GCs, within hours of administration (Capuron et al., 2003). The second mechanism involves cytokine mediated negative effects on GR function. Cytokines have been observed to induce a state of GC resistance through the down-regulation of GR function (A Miller, 2009). Under normal circumstances, crosstalk between cytokines and the GR involves the activation of signalling proteins that have been identified as targets for the anti-inflammatory actions of GCs (Zunszain, Anacker, Cattaneo, Carvalho, & Pariante, 2011). Cytokine activated signalling has been shown to impair GR function, through processes such as reducing GR translocation to the nucleus, as well as reducing overall GR expression (Engler et al., 2008; Pace, Hu, & Miller, 2007).

1.9.6. Inflammation and depression

There is a substantial amount of clinical evidence that supports the notion that the immune system is activated in depressed patients, and that this activation plays a role in disease progression as well as the ameliorating effects of antidepressant therapy (A Miller, Maletic, & Raison, 2009; Raison et al., 2006). Recent meta-analyses revealed that patients with MDD exhibit elevated peripheral blood inflammatory biomarkers, most commonly, proinflammatory cytokines IL-1, IL-6 and TNF-α, and CRP (Dowlati et al., 2010; Y Liu, Ho, & Mak, 2012). Levels of numerous other cytokines, such as IL-1 and monocyte chemoattractant protein 1 (MCP-1), have also been shown to be raised (Zorrilla et al., 2001). Furthermore, studies have demonstrated that such elevated levels of inflammatory biomarkers can be reversed by effective antidepressant treatment (Lanquillon, Krieg, Bening-Abu-Shach, & Vedder, 2000). Work from animal studies has also demonstrated that treatment with proinflammatory cytokines induces depressive-like behaviours in rodents (Anisman, 2009).

More direct evidence that inflammation is involved in the pathogenesis of depression comes from studies examining the effects of IFN-α treatment. It has been observed that within 3 months, up to 50% of patients undergoing such treatment acquire depression symptoms which meet criteria for a diagnosis of MDD (Musselman, Lawson, et al., 2001; Raison et al., 2005). These studies form the foundation for the theory of cytokine-mediated inflammatory processes in the pathogenesis of depression. Accordingly, inflammation is argued to contribute to a state of HPA axis dysfunction by promoting GC resistance (A Miller, 2009; Pace et al., 2007; Zunszain et al., 2011), through a process of cytokine-induced functional impairment of the GR (. Miller & Raison, 2006; Wang, Wu, & Miller, 2004).

1.9.7. Inflammation and early life stress

1.9.7.1. Prenatal maternal stress and offspring inflammatory response

As discussed in section 1.9.3.1, a large body of research has investigated the developmental plasticity effects of prenatal maternal stress on the foetus' neuroendocrine system. Given the observed integration between the HPA axis and the inflammatory response, as well as their mutual contribution to the risk of depression, interest has turned to the potential role of maternal-foetal inflammatory pathways as an associated candidate mechanism for the biological embedding of intrauterine stress (Entringer et al., 2012). Indeed, studies have demonstrated that stressed pregnant women not only show hyperactivity of the HPA axis (O'Keane et al., 2011), but also increased inflammation, indexed by higher levels of proinflammatory cytokines IL-6 and TNF- α (Coussons-Read, Okun, & Nettles, 2007; Coussons-Read, Okun, Schmitt, & Giese, 2005).

Studies investigating foetal programming of the immune response by maternal stress are few and far between. Currently, most work has been conducted in animals. In one study, pregnant mice were stressed, followed by an examination of cytokine levels in the brains of the pups who were sacrificed 24 hours after administration of lipopolysaccharide (LPS). Compared to pups of non-prenatally stressed damns, pups of prenatally stressed mothers exhibited elevated levels of IL-1β and TNF-α in the hippocampus (Diz-Chaves, Astiz, Bellini, & Garcia-Segura, 2013). In a study using non-human primates, the immune and GC response in offspring of prenatally stressed rhesus monkeys was compared to that of offspring of non-prenatally stressed mothers. Offspring of mothers who were prenatally stressed show elevated HPA axis activity, but a reduced inflammatory response (reduced levels of IL-6 and TNF-α) to LPS challenge. The authors argue that the reduced inflammatory response may be accounted for by the elevated HPA axis activity, given its suppressive effects on inflammation (Coe, 2002).

In a recent human study (*n* = 76), O'Connor and colleagues (O'Connor, Winter, et al., 2013) found that prenatal maternal anxiety predicted reduced adaptive immunity in the infant at 6 months. These effects were independent of obstetric and maternal factors. In a retrospective study (*n* = 34) into the effects of prenatal maternal stress on the offspring's inflammatory response, women who reported that their mother experienced emotional stress (major life events) during pregnancy, showed elevated levels of PBMC proinflammatory cytokines IL-4, IL-6 and IL-10 in response to phytohemagglutinin challenge, in comparison with women whose mothers were not prenatally stressed (Entringer, Kumsta, et al., 2008).

These studies suggest that HPA axis function and the inflammatory response are highly interlinked in both mother and the growing foetus. One potential mechanism for the effects of prenatal maternal stress on offspring inflammation could be through HPA axis-mediated effects. Indeed, if this were the case, it could be argued that hyperactivity of the HPA axis in the offspring could lead to GC induced immune-suppressive effects, which some of the aforementioned studies may have indeed indexed (Marques, O'Connor, Roth, Susser, & Bjørke-Monsen, 2013). Another non-mutually exclusive candidate mechanism could be the direct effect of elevated maternal-foetal inflammation during pregnancy, which may programme offspring immune reactivity. At present, there is limited evidence that directly supports this hypothesis.

1.9.7.2. Child maltreatment and inflammation

The link between child maltreatment and inflammation was first demonstrated in the Dunedin Multidisciplinary Health and Development Study (DMHDS). The DMHDS is a prospective longitudinal birth cohort study based in New Zealand that has followed over 1,000 individuals from early childhood to age 32. Danese and colleagues, including my second supervisor Prof Pariante (2007), showed that maltreatment in childhood predicted high inflammation, as indexed by plasma CRP levels at 32 years. This effect was independent of stress in adulthood,

health and lifestyle factors and co-occurring early life risks. Furthermore, CRP levels were observed to be highest amongst individuals who experienced current depression and had a history of child maltreatment (Danese et al., 2008).

More recently, in a retrospective study involving 1,000 US adults, cumulative early life stress, which included childhood experiences of abuse and neglect, was found to be positively associated with elevated CRP, IL-6 and adhesion molecules (Slopen et al., 2010). In a study (*n* = 69) involving depression free adults, individuals with a history of childhood maltreatment exhibited an elevated IL-6 response to a psychosocial stress challenge (Trier Social Stress Test), in comparison to non-maltreated individuals (Carpenter et al., 2010). These findings, it has been argued, reflect the biological embedding of early life stress through the inflammation processes, putting the individual at risk for inflammatory related psychiatric and cardio-metabolic diseases (Danese et al., 2009, 2011).

1.9.8. Metabolic syndrome

The metabolic syndrome is a clustering of metabolic risk factors, which predispose an individual to developing type II diabetes and CVD, in particular, coronary heart disease (CHD). CHD is one of the most common types of CVD. It is a condition characterised by plaque formation in the arterial walls through the pathological process of atherosclerosis. Atherosclerosis is an inflammatory condition in which the walls of the arterial blood vessels thicken as a result of accumulation of fatty deposits on the interior wall linings, specifically cholesterol (CHOL) and triglycerides (TGs; Maton, 1997). It constitutes a chronic inflammatory response in the walls of arteries whereby macrophages and white blood cells accumulate in the formation of plaques. This process is promoted by the amassing of low-density lipoproteins, responsible for the transport of CHOL and TGs, in the arteries. In contrast, high-density lipoproteins provide protective functional benefits, particularly by reducing arterial inflammation and plaque formation and ultimately protecting against atherosclerosis and subsequent CHD (Toth, 2005) There are various conceptualisations of the metabolic syndrome, as defined by various medical bodies, which differ in minor dimensions. Yet, there is a general consensus amongst all organizations that the primary components of metabolic syndrome comprise insulin resistance (IR), visceral obesity, high blood pressure and dyslipidaemia. IR is the condition whereby peripheral tissue cells fail to respond to insulin, produced by the pancreas. Insulin continues to be produced, yet the cells (muscle, fat and red blood cells) become resistant and are unable to use it effectively to absorb circulating glucose from blood plasma, leading to a state of hyperglycaemia. Beta cells in the pancreas subsequently increase their production of insulin to compensate, further contributing to hyperinsulinaemia. This condition of dysregulated glucose levels contributes to the development of type II diabetes (Beck-Nielsen, 2013)

Table 2 summarises the definitions according to the International Diabetes Federation (IDF; International Diabetes Federation, 2006), the World Health Organization (WHO; World Health Organization, 1999) and the European Group for the Study of Insulin Resistance (EGIR; Beck-

Nielsen, 1999). According to the WHO and the EGIR, IR is a crucial criterion for the diagnosis of metabolic syndrome, whilst more recent criteria from the IDF allow for diagnosis through the presence of central obesity, as an index of IR. The prevalence of metabolic syndrome is 6.7% among individuals aged 20 – 29 years, and rises to more than 40% in those 60 years or older (Ford, 2005). Although the metabolic syndrome is frequently referred to as a discrete entity, it is worth noting that, without a common aetiology, this grouping of metabolic abnormalities could reflect clustering of unrelated factors through to a constellation of indices for a shared pathological condition (Grundy, 2005).

Table 2. Diagnostic criteria for metabolic syndrome

	Organization		
	International Diabetes Federation	**World Health Organization**	**European Group for the Study of Insulin Resistance**
Diagnostic requirements	Central obesity (large WC or high BMI), plus at least two of the following: high FPG, high TGs, low HDL-C, high blood pressure.	Impaired glucose tolerance, impaired FPG or insulin resistance, plus at least two of the following : central obesity (large WHR or high BMI); dyslipidaemia (high TGs and low HDL-C); high blood pressure.	Insulin resistance plus at least two of the following: high FPG, dyslipidaemia (high TGs or low HDL-C); high blood pressure; central obesity.
FPG (nmol/L)	> 5.6	–	> 6.1
WC (cm)	> 94 (males) > 90 (females)	–	> 94 (males) > 80 (females)
WHR	–	> .90 (males) > .85 (females)	–
BMI (kg/m^2)	> 30	> 30	–
TGs (nmol/L)	> 1.7 nmol/L	> 1.7	> 2.0
HDL-C (nmol/L)	< 1.03 (males) <1.29 (females)	< .9 (males) < 1.0 (females)	< 1.0
Blood pressure (mmHg)	>130/85	> 140/90	> 140/90

Note. Adapted from (Beck-Nielsen, 1999; International Diabetes Federation, 2006; World Health Organization, 1999). FPG = fasting plasma glucose; WC = waist circumference; WHR = waist to hip ratio; TGs = triglycerides; BMI = body mass index; HDL-C = high density lipoprotein cholesterol. Blood pressure limits are systolic/diastolic.

1.9.9. Metabolic syndrome and the HPA axis and inflammation

The HPA axis, inflammation and metabolic function are intimately linked. In a study of 205 men, HPA axis activity (enhanced responsiveness to ACTH) was positively associated with raised blood pressure, glucose intolerance, and hypertriglyceridemia (Reynolds et al., 2001). Notably, low birth weight was also observed to be a correlate of enhanced HPA axis activity, alluding to the notion that HPA hyperactivity may be a mediating mechanism between an adverse intrauterine environment and later metabolic abnormalities. Elevated GCs are thought to increase plasma glucose indirectly through their antagonising effects on insulin action. They have also been observed to promote the action of lipoprotein lipase, which modulates the storage of fat (TGs and CHOLs) in adipocytes (fat cells), and thus visceral fat deposition (Dinan, 2004).

One argument for the link between metabolic syndrome and inflammation is derived from the finding that proinflammatory cytokines are overexpressed in obese individuals by adipose tissue (Hotamisligil, Shargill, & Spiegelman, 1993). The phenomenon is argued to originate in saturated adipocytes that respond to overfeeding by the release of proinflammatory cytokines, specifically TNF-α and IL-6. This localised inflammation can trigger systemic inflammation which is associated with elevated parameters of the metabolic syndrome such as hypertension, dyslipidaemia and IR (Emanuela et al., 2012). Furthermore, the energy demand of the activated immune response has been causally related to the metabolic syndrome (Straub, 2011). Also, elevated CRP has become a widely acknowledged risk marker for CVD (Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker, Hennekens, Buring, & Rifai, 2000; Ridker, Rifai, Rose, Buring, & Cook, 2002), with a strongly evidenced association with atherosclerosis. It has also been posited that poor metabolic function, such as IR, could indirectly elevate systemic inflammation through resistance to the anti-inflammatory actions of insulin (Esposito & Giugliano, 2004). Thus, it is conceivable that once in a state of persistent inflammation, HPA axis activation or metabolic dysfunction, any of these conditions could have positive feedback on one another, leading to a state of cumulative activation across systems (Leonard, 2013).

1.9.10. Metabolic syndrome and depression

Research into the association between metabolic syndrome and depression is rooted in the established finding of high comorbidity between CVD and depression (Barth, Schumacher, & Herrmann-Lingen, 2004; Fielding, 1991). This association is believed to be bidirectional, with cohort studies demonstrating a positive association between depressive symptomatology and CVD mortality (Wulsin et al., 2005), as well as the elevated prevalence of depression in CVD patients (Fielding, 1991; Whooley & Wong, 2013). Metabolic syndrome, amongst other processes, such as inflammation, HPA axis hyperactivity, health behaviours, and platelet hyperactivity, is argued to be a key pathophysiological factor linking depression to adverse cardio-metabolic outcomes (Celano & Huffman, 2011; Halaris, 2013; Whooley & Wong, 2013).

Indeed, in a cross-sectional study in Australia involving 1,690 participants, Dunbar and colleagues (2008) observed that individuals diagnosed with metabolic syndrome had significantly greater depressive psychopathology. Moreover, in a subgroup of diabetes-free individuals, this association remained. Furthermore, low HDL-C levels and large waist circumferences (WCs) showed significant independent associations with depression. In a US sample of over 6,000 CVD and diabetes-free adult men and women, the prevalence of metabolic syndrome was significantly elevated amongst women with a history of depression. This effect remained when controlling for age, education, smoking, physical inactivity, carbohydrate consumption, and alcohol use. No effect was observed in men, however (Kinder, Carnethon, Palaniappan, King, & Fortmann, 2004). In a recent systematic review involving over 40 cross-sectional and cohort studies, support for bi-directional effects between the metabolic syndrome and depression were observed, with the majority of studies reporting depression as the outcome, whereby effect sizes ranged between 1.07 - 1.91 odds ratio (Pan et al., 2012). Furthermore, a meta-analysis into the relationship specifically between obesity and depression revealed reciprocal links (Luppino et al., 2010).

1.9.11. Metabolic syndrome and early life stress

1.9.11.1. Metabolic syndrome and exposure to prenatal maternal stress

As discussed in section 1.3, the *in utero* environment presents a unique developmental time period in which the offspring's developing organs are particularly susceptible to plasticity effects in response to intrauterine adversity – the FOAD hypothesis. Exposure to suboptimal maternal hormonal states and under-nutrition during pregnancy are the most commonly researched forms of prenatal adversity.

The link between prenatal maternal adversity and offspring metabolic syndrome, research has focused primarily on the association between under-nutrition, often indexed through low birth weight, and components of the metabolic syndrome, such as hypertension, dyslipidaemia and IR, given their centrality to the development of CVD and type II diabetes. As mentioned, some of the earliest investigations into this link were the work of Barker and colleagues (D Barker, Gluckman, et al., 1993; D Barker, Hales, et al., 1993), who demonstrated in a cohort of British adults, that low birth weight significantly predicts hypertension, impaired glucose tolerance and dyslipidaemia. Research has also indicated elevated adulthood BMI in offspring born to mothers who experienced famine during pregnancy (Ravelli et al., 1998).

Such associations are argued to be a consequence of alterations to physiological responsiveness and morphological changes as a function of a varying gestational environment during sensitive periods of development. Plasticity in peripheral targets, such as adipose tissue, the pancreas and liver, in addition to the brain and component neurobiological systems (e.g. HPA axis programming, inflammation programming), have been proposed as candidate mechanisms by which programming of metabolic dysfunction occurs, given their collective role in metabolic regulation after birth (Entringer et al., 2012), see Figure 1. Metabolic related diseases are believed to arise through a series of interactions between a vulnerable predisposition, as

programmed during foetal life, and environmental influences after birth (D Barker, 2008; Rinaudo & Wang, 2012). Indeed, metabolic diseases are most common in low- and middle-income countries where the combination of foetal under-nutrition and adulthood over-nutrition are most common (Symonds, Sebert, Hyatt, & Budge, 2009).

Less research has explored specifically the association between prenatal maternal psychosocial stress and offspring metabolic syndrome and its components. One reason for this may be due to the fact that many prospective studies into the effects of prenatal emotional stress have only been set up in the past two decades, and as clinical abnormalities in metabolic parameters are most prevalent in older adults, a lack of observations may reflect prematurity in the potential manifestation of effects, rather than non-existence of such effects. Nevertheless, in a prospective Dutch cohort study involving over 2,000 mother-child pairs, higher prenatal maternal cumulative stress (depressive symptoms, anxiety, daily hassles, job strain) was positively associated with systolic and diastolic blood pressure and mean arterial pressure, in the offspring at age eight (van Dijk, van Eijsden, Stronks, Gemke, & Vrijkotte, 2012).

In a retrospective study involving 58 young adult offspring, reports of maternal severe life events during pregnancy (death of someone close, severe illness of someone close, relationship conflicts, severe financial problems, car accidents, becoming political refugees) were significantly associated with elevated two-hour insulin following an oral glucose tolerance test, suggesting glucose-insulin metabolism irregularities (Entringer, Wüst, et al., 2008). These differences were independent of factors related to IR such as, birth weight, gestational age, a family history of diabetes, gestational diabetes, BMI, smoking and inflammatory conditions. Furthermore, offspring of mothers who reported greater prenatal life events had lower HDL-C levels as well as higher BMIs, in comparison with offspring of mothers who didn't report any life events during pregnancy. Whilst these studies encompass methodological limitations and proxy measures of the metabolic syndrome, their findings support the notion that foetal exposure to maternal

psychosocial stress during pregnancy can have adverse effects on cardio-metabolic parameters later in life. Tests using prospective designs reaching further along the offspring's lifespan are required to replicate such effects.

In an interesting rodent study, the effects of prenatal stress on pup atherosclerosis and inflammation were examined using an apolipoprotein E-deficient (apoE$^{-/-}$) mouse strain, which is a well-established mouse model of hyperlipidaemic-induced atherosclerosis (Ho et al., 2013). Ho and colleagues stressed pregnant apoE$^{-/-}$ dams and examined for the presence of atherosclerotic plaques, as well as cytokine secretion in stimulated splenocytes in the offspring at 15 weeks. ApoE$^{-/-}$ offspring of prenatally stressed dams exhibited more large atherosclerotic plaques, as well as raised levels of TNF- α in comparison with apoE$^{-/-}$ pups of non-prenatally stressed dams. The authors argue that this finding illustrates an inflammatory-mediated effect of prenatal stress on atherosclerosis. Notably, this study also provides evidence for the link between prenatal stress and inflammation, and points to an effect on system inflammation. Yet, the precise mechanisms by which this programming may occur remain elusive. Candidate pathways could include specific programming of metabolic-regulating body organs, as well as programming of neurobiological systems (HPA axis and inflammatory response).

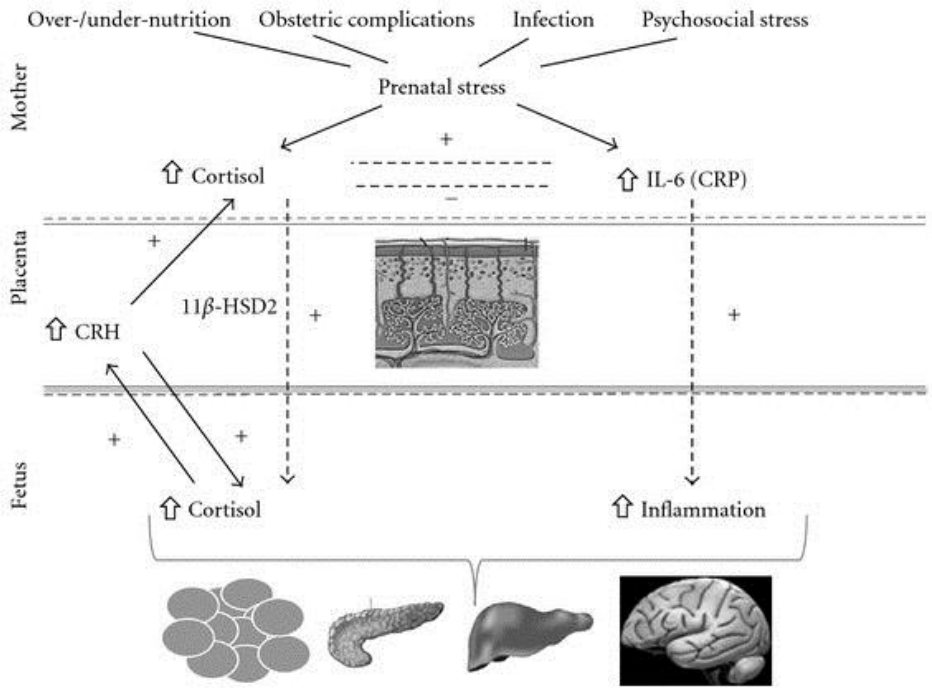

Figure 1. Potential targets for foetal programming of metabolic syndrome parameters, adapted from Entringer et al., 2012

1.9.11.2. Metabolic syndrome and child maltreatment

In the past decade there has been an explosion of interest in the relationship between childhood maltreatment and physical health problems (Norman et al., 2012). Specific to the metabolic syndrome, a large number of studies have investigated the effect of childhood maltreatment on obesity and other metabolic parameters, many in the context of risk for CVD. These studies have included retrospective and prospective epidemiological studies. For example, in a retrospective cohort study of 13,177 US adults, reports of CPA predicted obesity (Williamson, Thompson, Anda, Dietz, & Felitti, 2002). In a meta-analysis into the effects of CSA that included 31 studies, an overall effect of a history of CSA on elevated adulthood BMI was observed (Irish, Kobayashi, & Delahanty, 2010). Furthermore, in a recent meta-analysis conducted into the effects of CPA, emotional abuse and neglect, which included 124 studies, a significantly increased risk for obesity was observed for CPA and emotional abuse (Norman et al., 2012). Finally, in a prospective longitudinal study involving 8,471 US adolescent who have been followed into young adulthood, child neglect was associated with a faster than average rate of BMI growth over time (Shin & Miller, 2012).

In a prospective longitudinal study of 9,310 British adults, a history of child maltreatment was associated with elevated glycosylated haemoglobin (HbA1c) and obesity in middle adulthood (Thomas, Hyppönen, & Power, 2008). In a longitudinal US study involving 342 middle aged women, a history of childhood maltreatment predicted incident metabolic syndrome over the course of seven years (Midei, Matthews, Chang, & Bromberger, 2013). Furthermore, in a study of 95 first-episode psychosis patients, childhood maltreatment was significantly associated with increased TG levels compared to controls (Hepgul et al., 2012).

These studies demonstrate the link between various types of child abuse and neglect and components of the metabolic syndrome. Key proposed pathways from child maltreatment to

metabolic ill health include chronic inflammation and maladaptive eating patterns (Dallman et al., 2003; Danese et al., 2009).

1.10. Aims and hypotheses

1.10.1. Aims of the study

The literature reviewed above has shown clear evidence for an association between offspring exposure to prenatal maternal stress and psychopathology in childhood. The best evidence for this association has come from prospective longitudinal studies. However, no prospective study has yet found evidence for an effect of prenatal maternal depression on offspring affective psychopathology in adulthood. Moreover, findings from current research have also shown a robust and reliable link between child maltreatment and depression in adulthood. The primary aim of this study is to investigate whether offspring exposure to prenatal maternal depression predicts offspring depression in young adulthood, and whether offspring experience of child maltreatment cumulatively adds to this effect.

The second aim of this study is to characterise the physiological characteristics of young adult offspring who were exposed to prenatal maternal depression, in terms of HPA axis function, inflammation and metabolic function. The reviewed research has shown that abnormalities in all three systems are associated with exposure to prenatal maternal stress, gross psychosocial adversity during childhood, and depressive psychopathology. The purpose of this investigation is to gain a better understanding of whether dysregulation in these three systems could be interlinked mechanisms for the biological embedding of early life stress. These investigations will also cast light on whether gestational stress and childhood stress (i.e. child maltreatment) effect their impact through the same putative biological pathways.

1.10.2. Hypotheses

1.10.2.1. Depressive psychopathology

1) Exposure to prenatal maternal depression predicts depression in young adulthood (18-25 years).
2) This effect of prenatal maternal depression is independent of environmental adversities occurring during childhood (birth-17 years).
3) Experience of childhood maltreatment modulates the effect of offspring exposure to prenatal maternal depression on depression in young adulthood.

1.10.2.2. Reactivity in biological systems

1.10.2.2.1 HPA axis, inflammation and parameters of the metabolic syndrome

4) Alterations in cortisol levels, inflammation and metabolic parameters at 25 years will be positively associated.

1.10.2.2.2 HPA axis

5) Offspring exposed to maternal depression *in utero* will have an altered diurnal cortisol profile at 25 years.
6) Offspring exposed to prenatal maternal depression and who are depressed in adulthood will have the greatest alterations in cortisol levels at 25 years.
7) Offspring exposed to childhood maltreatment will show similar alterations in cortisol levels to offspring exposed to prenatal maternal depression.

1.10.2.2.3 Inflammation

8) Offspring exposure to depression *in utero* will be associated with elevated inflammation at 25 years.
9) Cumulative experience of stress *in utero* (exposure to prenatal maternal depression) and depression in adulthood will result in the greatest inflammation levels.
10) Offspring exposed to childhood maltreatment will also exhibit elevated inflammation.

1.10.2.2.4 Parameters of the metabolic syndrome

11) Offspring exposed to depression *in utero* will exhibit metabolic abnormalities
12) Offspring exposed to prenatal maternal depression and who experience depression in adulthood will have the greatest abnormalities in metabolic function.
13) Offspring exposed to childhood maltreatment will also exhibit abnormalities in metabolic function.

CHAPTER 2: METHODS

2.1. Design

This thesis was conducted as a continuation of the South London Child Development Study (SLCDS; 1986-2003), which formed a framework for my investigation. The SLCDS is a prospective longitudinal birth cohort study that was setup in 1986 as a PhD project by Prof D Sharp (D Sharp, 1992). The original purpose of the SLCDS was to assess the prevalence of women's mental health problems in the perinatal period. Since, the SLCDS evolved into a longitudinal study of the offspring and their families through childhood (4, 11 and 16 years). This PhD project has continued the SLCDS by assessing the offspring at 25 years.

2.1.1. Contribution of work by the candidate

It was my responsibility to conduct and coordinate the follow-up of the offspring at 25 years under the supervision of Dr S Pawlby (Primary Supervisor and Lecturer, Institute of Psychiatry, King's College London [KCL], UK) and Prof C Pariante (Secondary Supervisor and Professor, Institute of Psychiatry, KCL, UK). I was responsible for obtaining ethical approval for the follow-up and also contributed to the study design. A trained research assistant (RA) helped with the tracing of participants and also interviewed 18 (17.5%) of the offspring, whilst I interviewed the rest. Phlebotomy staff at the National Institute for Health Research – Wellcome Trust Clinical Research Facility at King's College Hospital (KCH) assisted with the collection of blood samples. Staff at KCH Phlebotomy Department processed and assayed all blood samples. Dr P Zunszain (Lecturer, Institute of Psychiatry, KCL, UK) carried out all of the salivary cortisol assays. I was responsible for all data entry and coding. Dr T Chua (Visiting Psychiatrist, Institute of Psychiatry, KCL, UK) was consulted for the rating of psychiatric diagnoses. I conducted all of the statistical analyses.

2.2. Sample

2.2.1. SLCDS (1986-2003)

All pregnant women who approached either of two South London National Health Service (NHS) General Practitioner (GP) clinics ([i] Lambeth Road Group Practice (LRGP), SE11 6SP; [ii] Gallions Reach Health Centre (GRHC), SE28 8BE) for antenatal care between January 1st 1986 and December 31st 1986 were invited to take part in the SLCDS. Overall, 395 pregnant women presented for antenatal care across the two clinics. Three hundred and seventeen women were deemed eligible for participation.

Two hundred and fifty-two women participated in the first assessment visit at 20 weeks pregnant. A further three interview assessments were scheduled to take place at 36 weeks pregnant, 3 months postnatal and 12 months postnatal. However, due to time constraints, a 75% randomised subsample[1] were selected for interview at 36 weeks pregnant and 3 months postnatal, with the remaining 25% completing postal self-report questionnaires only. The random sample did not differ statistically in any sociodemographic or clinical characteristics in comparison with the non-random sample (D Sharp, 1992). A total of 203 (80.6%) women completed the study at 12 months postnatal, 149 of these being of the random sample.

Follow-up assessments were arranged at the offspring's 4th, 11th and 16th birthdays. At 16 years, only participants of the random sample were given a full interview. One hundred and twenty-seven offspring were fully interviewed at 16 years.

[1] Hereon referred to as "the random sample".

2.2.2. Present study: 25-year follow-up (2012-13)

For the 25-year assessment, only offspring of the random sample whose mothers agreed to take part in follow-up phases after the original perinatal phase were sought for participation (*n*= 151). One hundred and three offspring participated. One hundred and one had been seen previously at 16 years, whilst two had been unavailable at 16 years but were previously seen at 11 years. This represents a 79.5% retention rate of the random sample based on offspring who participated at 16 years.

Of the 48 offspring who did not participate, reasons for non-participation were as follows: 16 (33.3%) had left the study before the adolescent follow-ups, 8 (16.7%) were not able to be contacted, 19 (39.6%) declined to participate (hard refusal) and 5 (10.4%) initially agree to participate but evaded further contact (soft refusal). Figure 2 depicts the progress of participation for the random sample from SLCDS onset to 25 years.

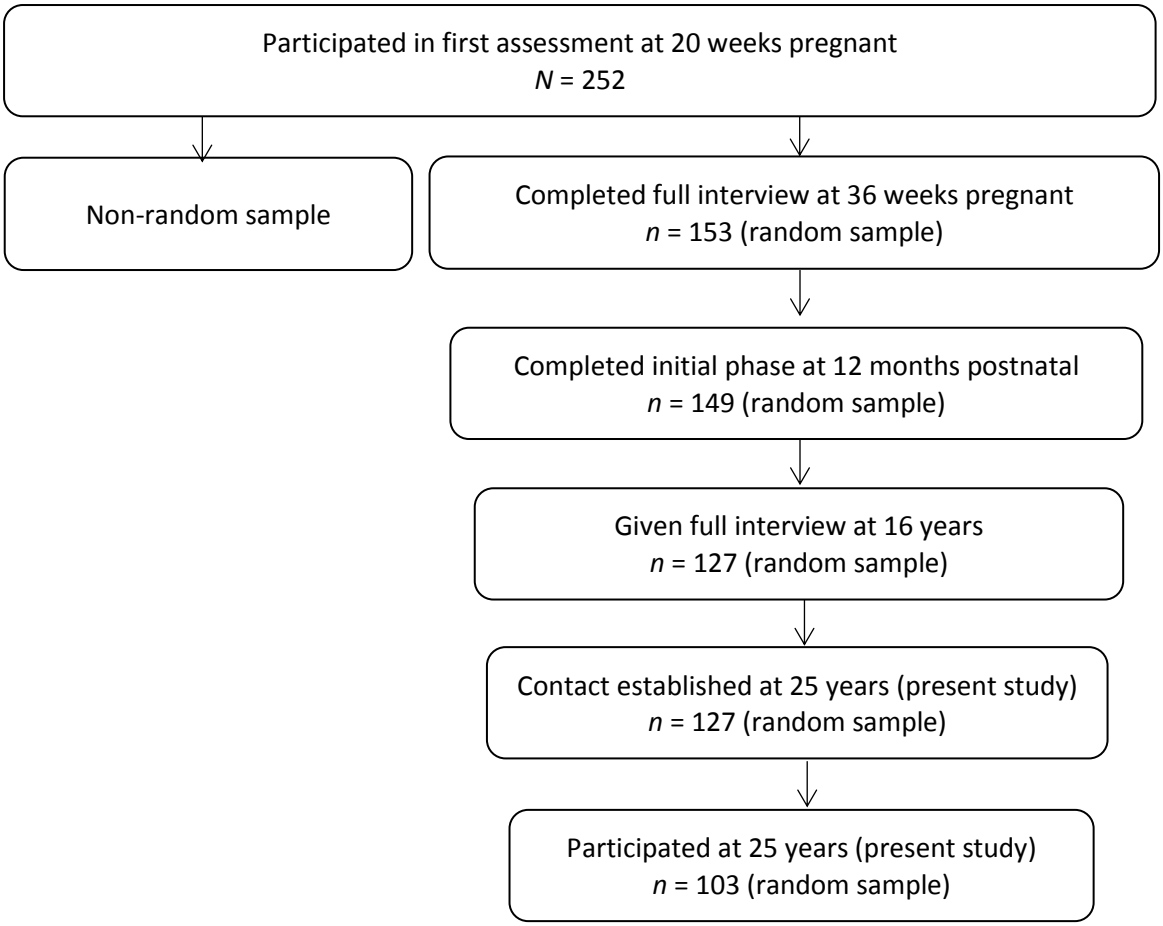

Figure 2. Flow chart of participation in the current follow-up at 25 years

2.3. Procedure

2.3.1. SLCDS (1986-2003)

2.3.1.1. Ethics

Ethical approval was received from the University of London. At the SLCDS onset, informed consent was obtained from the pregnant women attending LRGP and GRHC to participate in the initial 20-week assessment. A complete description of the study was provided prior to consent being sought.

2.3.1.2. Perinatal phase

Maternal assessments occurred at 20 and 36 weeks of pregnancy and at 3 and 12 months postnatal. Each visit was conducted in the mother's home by one of two academic GPs, who underwent training in the assessments specific to the study at the Institute of Psychiatry. The GPs only assessed participants who were patients at the practice where they did not work. At the initial 20-week assessment, full sociodemographic and clinical information was collected. At further time points current mental health and family information was updated. In addition, RAs collected mental health related information from the clinical medical notes and the health visitors' records at 12 months postnatal.

2.3.1.3. Four, 11 and 16 year phases

The families were visited at home at 4, 11, and 16 years. At 11 and 16 years, two interviewers who were unaware of the information collected at previous visits, interviewed mothers and offspring separately. All interviewers held at least a bachelor's degree in psychology, and were further trained in the assessments specific to the project at the Institute of Psychiatry, King's

College London (KCL). At each time point, mental health, sociodemographic, life history and relationship information, current and retrospective to the last visit, was collected. The offspring's schools were also contacted at 16 years to record final GCSE examination results. Mothers and offspring individually consented to be traced for future follow-ups.

2.3.2. Twenty-five year phase (2012-13)

2.3.2.1. Ethics

Ethical approval for the 25-year offspring phase was obtained from London – Camberwell St Giles National Research Ethics Service Committee (reference number: 11/LO/0812).

2.3.2.2. Tracing and interview

All random sample offspring whose mothers had consented to be traced at previous follow-ups were sought. A letter was posted to the mother at her last-known address requesting her current telephone contact details. In cases where the offspring was not living with the mother at the last visit (i.e. 16 or 11 years), letters were sent to the guardian at that time (e.g. father, aunt). Once contact was made, mothers were telephoned to explain that the next phase of the study was being conducted and were asked to provide their offspring's contact details. In cases where no letters were returned, the Data Linkage and Extract Service of the Health & Social Care Information Centre was employed. Current GP details for untraced mothers and offspring were requested. GPs were then contacted via post and asked to forward the letters to the mothers and offspring at their currently known address. Tracing was conducted by myself and a RA.

Once telephone contact was made with the offspring, a full description of the study was provided followed by an invitation to come for the assessment at the National Institute for

Health Research – Wellcome Trust Clinical Research Facility at KCH. Participants who were unable to travel to KCH were visited in their home. A detailed information sheet (Appendix A) was reviewed with the participant, followed by the gaining of informed consent (see Appendix B) prior to the commencement of the interview. The interview comprised a combination of questionnaires and semi-structured interview assessments. All interviews were audio recorded, and sociodemographic, mental health and life history information was collected. A RA trained at the Institute of Psychiatry, KCL, assisted with the interviewing of some of the participants. All interviewers were blind to participants' information as collected at previous assessments. For the final part of the assessment, offspring were asked to have their height, weight and waist ratio measured and to provide a blood sample. In cases where I did not interview the participant, a trained phlebotomist from KCH collected the blood samples. All blood samples were delivered to KCH Phlebotomy Department within 15 minutes of sample collection. In cases where the participant was interviewed at home, samples were transported on ice and deposited as quickly as possible. To end the interview, participants were provided with a set of six Salivette swabs (Sarstedt, Leicester, UK) to take home in order to provide saliva samples over the course of one day. They were provided with a prepaid special delivery package to return the samples. Finally, participants were paid £50 for their time and thanked. Participants were given reminders to collect their saliva samples via text. Once the saliva samples had been received in the post, they were emailed a further electronic £10 Amazon.co.uk voucher.

2.4. Measures

2.4.1. Maternal mental health

2.4.1.1. Prenatal maternal depression

A variable was constructed to capture all instances of clinically relevant depression during pregnancy. At 20 and 36 weeks, pregnant mothers were interviewed using the Clinical Interview Schedule (CIS; Goldberg, Cooper, Eastwood, Kedward, & Shepherd, 1970). International Classification of Diseases 9th Revision (ICD-9) diagnoses of women's current mental state over the past two weeks were rated. Criteria for the following depressive disorder diagnoses were met: neurotic depression (300.4); a brief depressive reaction (309.0); a prolonged depressive reaction (309.1); depression – other (311); depressive personality (301.1). The most common diagnosis was neurotic depression. The overall agreement between the two interviewers of the reported symptoms on the CIS based on nineteen tape-recorded interviews, given as a weighted kappa coefficient, was .81. Twenty-three (22.3%) women were rated as meeting criteria for a depressive disorder at 20 weeks pregnant, and twenty-three were classified as depressed at 36 weeks pregnant (22.3%). A dichotomous variable was created that detailed whether a mother had been clinically depressed at either time point in pregnancy (D Sharp, 1992).

2.4.1.2. Prenatal maternal anxiety

Mothers completed the Leeds Anxiety Scale (Snaith, Bridge, & Hamilton, 1976) at 20 and 36 weeks pregnant. Scores on the anxiety subscale at each time point were averaged to provide an index of overall anxiety symptoms during pregnancy.

2.4.1.3. Maternal depression from birth to 16 years

At 3 and 12 months postnatal mothers were interviewed about their current mental state (past two weeks) using the CIS, from which ICD-9 depressive disorder diagnoses were rated (300.4, 309.0, 309.1). Postnatal depression was defined by the combined ICD-9 diagnoses from the 3- and 12-month interviews; if the mother had met ICD-9 criteria for a depressive disorder at either time, she was rated as having experienced postnatal depression (birth to 1 year). At 4, 11 and 16 years mothers were interviewed about their current mental state and experience of depressive symptoms retrospective to the previous assessment using the lifetime version of the Schedule for Affective Disorders and Schizophrenia (SADS-L; Spitzer, Endicott, & Robins, 1978). At these assessments diagnoses of major (03), minor (13) and intermittent (14) depressive disorder were made in consultation with the lead psychiatrist on the team using Research Diagnosis Criteria (RDC). The current and ''retrospective to last visit'' data were used to assess the occurrence of maternal depression over the child's lifetime. Variables were created to measure the children's exposure to maternal depression in early (between 1 and 4 years), middle (between 4 and 11 years) and late chilldhood (between 11 and 16 years). For each time interval, depression was rated if a mother met criteria for a diagnosis currently at the time of interview or in the period retrospective to the last visit. Mothers were also interviewed retrospectively using the SADS-L about their mental state during the entire first postnatal year, from which RDC diagnoses of depression across the 1st postnatal year were generated. A dichotomous variable of whether the mother had ever experienced depression in the period between the child's 1st and 16th birthday was also generated.

2.4.1.4. Maternal previous psychiatric history

Information on a mother's personal history of psychiatric problems (depression, anxiety, substance use problems, psychotic disturbances) prior to pregnancy was collected using a clinical schedule at 20 weeks pregnant. A dichotomous measure of mothers' personal histories of psychiatric problems prior to the index pregnancy was generated (0 = no history; 1 = history).

2.4.2. Maternal sociodemographic characteristics

2.4.2.1. Maternal age

The mother's age at the birth of the birth of the index child was recorded in whole years.

2.4.2.2. Maternal education

Information on a mother's highest educational achievement was obtained at 20 weeks pregnant. A dichotomised variable of basic qualifications (O-levels) or higher (0) versus no qualifications (1) was generated.

2.4.2.3. Maternal social class

Information about a mother's occupation was collected at 20 weeks pregnant, and was used to make ratings about their social class according to Goldthorpe and Hope's (1974) grading of occupations. The highest ranked employment a mother had ever held was used to determine the mother's socioeconomic status. Mothers were classified into middle class (0) versus working class (1).

2.4.2.4. Maternal ethnicity

Mothers' reports of their cultural backgrounds were dichotomised into White British (0) versus not White British (1).

2.4.2.5. Maternal marital status

Mothers reported their marital status at 20 weeks pregnant. A dichotomous variable of whether a mother was married (0) versus unmarried (1) was generated. This distinction was selected based on the fact that most women with a long-term partner were married in the 1980s. Thus, this distinction is believed to index partnership presence.

2.4.3. Measures of mothers' perinatal behaviours

2.4.3.1. Maternal smoking during pregnancy

During the second- and third-trimester interviews, mothers reported the number of cigarettes smoked per day. Composite measures of prenatal smoking were constructed by averaging the scores reported at each interview

2.4.3.2. Maternal drinking during pregnancy

Mothers reported the number alcohol units drunk per week at the second- and third-trimester interviews. A composite measure of prenatal drinking was generated by averaging the scores reported at each interview.

2.4.3.3. Medical problems during pregnancy

At the third-trimester interview mothers reported the number of medical problems they had during the pregnancy, ranging from none, a few to a lot. Reports were recoded into none (0) versus some (1).

2.4.3.4. Breastfeeding

The length of breastfeeding (in weeks) was ascertained by questionnaire at eight months postnatal.

2.4.4. Obstetric outcomes

2.4.4.1. Offspring birth weight

Measures of offspring birth weight were obtained from medical records of the birth and were recorded in grams.

2.4.4.2. Offspring gestation age

Length of gestation was recorded in whole weeks rounded up or down to the nearest week.

2.4.5. Offspring depression in adulthood

At 25 years, offspring were interviewed about their current mental state (past month) and experience of depressive symptoms retrospective to the previous assessment using the Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV; First, Spitzer, Gibbon, & Williams, 1996; Appendix F). DSM-IV diagnoses of mood disorders in adulthood were rated in conjunction with the psychiatrist on the team. Diagnoses were of major depressive disorder (MDD), single episode (296.2x), recurrent episode (296.3x), depressive disorder not otherwise specified (NOS; 311) and dysthymic disorder (300.4). A dichotomous variable was created to index adulthood DSM-IV depression based on whether an offspring had met criterion for any of the above depressive disorders since their 18th birthday. A continuous variable was also constructed from the highest number of DSM-IV depressive symptoms reported in any given episode.

Furthermore, offspring concurrent depressive symptomatology in the week preceding the 25-year interview was recorded using the Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960; Appendix G). Questions 1-17 were rated and a total depressive symptomatology score generated. Scores of 8 or above were classified as reflecting significant depressive symptomatology.

2.4.6. Offspring experience of childhood maltreatment

Measures of offspring experience of sexual abuse, physical abuse, emotional abuse and neglect were obtained. Emotional abuse and neglect were measured using the Childhood Experience of Care and Abuse Questionnaire (CECA.Q; Bifulco, Bernazzani, Moran, & Jacobs, 2005; Smith, Lam, Bifulco, & Checkley, 2002; Appendix H) administered to the offspring at 25 years. Physical and sexual abuse were rated through a combination of offspring reports provided at 25 years using the CECA.Q, plus offspring and parental reports of lifetime instances of sexual and physical abuse provided at 11 and 16 years using the Child and Adolescent Psychiatric Assessment (CAPA; Angold & Costello, 2000; Appendix I). Maltreatment was rated if any one of the three types of abuse (physical, sexual, emotional) or neglect were ever present (0 = non-maltreated, 1 = maltreated)

2.4.6.1. Emotional abuse and neglect (CECA.Q)

Emotional abuse was indexed through offspring ratings of severe parental antipathy, whilst neglect reflected experience of severe parental neglect. Ratings were based on experiences that occurred up to 17 years of age. Scoring of the antipathy and neglect scales on the CECA.Q were conducted in accordance with the guidelines published by Bifulco and colleagues (2005), whereby the most conservative cut-off points were selected in order to ensure that only severe instances of abuse and neglect were counted, thus minimising the possibility of false positives.

Total scores for maternal and paternal antipathy were calculated. Maternal antipathy scores of 7-27 were recoded into 0 (minimal antipathy) whilst scores ≥ 28 were recoded as 1 (severe antipathy). Paternal antipathy scores of 7-29 became 0 (no/non-severe antipathy) whilst scores of 30 or more were transformed into 1 (severe antipathy). Total scores for maternal and paternal neglect were calculated. Emotional abuse was rated if severe antipathy was experienced from either parent (0 = no/minimal emotional abuse; 1 = emotional abuse).

Maternal neglect scores of 8-24 were then recoded into 0 (minimal neglect) whilst scores of 25 or more were recoded as 1 (severe neglect). Paternal neglect scores of 8-25 became 0 (minimal neglect) and scores of 26 or more were transformed into 1 (severe neglect). Neglect was rated if severe neglect was experienced from either parent (0 = no/minimal neglect, 1 = neglect).

2.4.6.2. Physical and sexual abuse (CECA.Q and CAPA)

At 25 years, offspring reported retrospectively on their experience of sexual and physical abuse up to age 17 using the CECA.Q. Physical abuse from maternal and paternal figures was rated separately by summing the relevant responses: (i) more than once (1), only once (0); (ii) hit with a belt/stick or punched/kicked (1), hit with hand/other (0); (iii) resulted in injury/bruising (1), no injuries (0); (iv) perpetrator out of control (1), in control (0). This resulted in scores ranging from 0 to 4 for each parental figure. Scores of 0-2 were further recoded into 0 (minimal physical abuse), whilst scores of 3-4 were coded as 1 (severe physical abuse). CECA.Q parental abuse was then rated if an offspring received a score of 1 from either parent (0 = no/minimal physical abuse, 1 = physical abuse).

Experiences of sexual abuse (as assessed at 25 years through the CECA.Q) were rated separately for each unwanted sexual experience. A total score for each unwanted sexual experience was calculated by adding together the responses for the following items: (i) perpetrator known (1), not known (0); (ii) perpetrator relative (1), not relative (0); (iii) perpetrator lived in household (1), lived outside household (0); (iv) more than once (1), only once (0); (v) perpetrator touched child's private parts (1), didn't involve this (0); (vi) involved touching perpetrator's private parts (1), didn't involve this (0); (vii) sexual intercourse (1), not sexual intercourse (0). The possible range of scores for each unwanted sexual experience therefore ranged from 0 to 7. In order to create a dichotomised sexual abuse variable, firstly scores of 0 or 1 for each unwanted sexual experience were recoded as 0 (minimal sexual abuse) and scores of 2-7 were coded as 1 (severe

sexual abuse). A rating of 1 for either or both the first or second unwanted sexual experience dichotomised variables was rated as indicating CECA.Q sexual abuse and coded as 1 (severe sexual abuse) whilst ratings of 0 for both experiences were coded as 0 (minimal sexual abuse).

At 11 and 16 years offspring and the primary caregiver (in most cases the mother) provided independent reports about any lifetime experiences of sexual or physical abuse up to that time point (Appendix I). Physical abuse was rated if respondents reported incidents of abuse that involved at least some physical injury or force with potential for such. Sexual abuse was defined as incidents in which a perpetrator involved the offspring in activities for the perpetrator's own sexual gratification. This could include fondling, oral contact, genital or anal intercourse. CAPA physical and sexual abuse were rated, respectively, if either offspring or parents reported incidents of physical or sexual abuse at 11 or 16 years that met criterion for the CAPA definition (0 = no/minimal physical abuse, 1 = physical abuse; 0 = no/minimal sexual abuse; 1 = sexual abuse).

Finally, physical/sexual abuse was classified by the combined CECA.Q and CAPA ratings; if physical/sexual abuse was rated in either measure, physical/sexual abuse was rated, respectively (0 = no/minimal physical abuse, 1 = physical abuse; 0 = no/minimal sexual abuse; 1 = sexual abuse).

2.4.7. Offspring recent stressful life events in adulthood

Offspring recent life events were rated in order to give a measure of stress in adulthood. At 25 years, offspring completed a life events questionnaire (Appendix E), which detailed life events occurring in the last 6 months, along with the level of distress attached to the event (0 = not distress, 1 = mildly distressing; 2 = moderately distressing; 3 = severely distressing). A composite variable was created which summed the total number of severely distressing (level 3) life events in the 6-month period preceding the interview.

2.4.8. Offspring personal characteristics at 25 years

A scheduled was devised to collect offspring personal characteristics at 25 years (Appendix C). Information on current marital status, children, housing, employment, cultural background and education was collected. Dichotomous variables of the following constructs were then generated.

2.4.8.1. Offspring ethnicity

Self-reported cultural background was dichotomised into White British (0) versus not White British (1).

2.4.8.2. Offspring education

Offspring reported on their highest level of educational achievement. In cases where offspring reported only GCSEs, corroborative information on the nature of these qualifications was obtained from the information provided by the offspring's school at 16 years. Where there was discrepancy between offspring reports and school reports, the school reports were used. Offspring were dichotomised into those with some qualifications (at least one GSCE grade A*-C; 0) versus those with none (1).

2.4.9. Offspring lifestyle factors at 25 years

Lifestyle information was collected via a schedule devised for the study at 25 years (Appendix D). This included information on current smoking, drinking, physical fitness and eating habits, as well as medication use. The following variables were then computed.

2.4.9.1. Offspring smoking

A continuous variable of offspring current smoking habits was created as follows: 0 = non-smoker; 1 = light smoker (1-9 cigarettes per day); 2 = moderate smoker (10-19 cigarettes per day); 3 = heavy smoker (20+ cigarettes per day).

2.4.9.2. Offspring alcohol consumption

Offspring current average drinking consumption was coded as follows: 1 = drinks less than once per week; 2 = drinks 1-2 days per week; 3 = drinks 3-4 days per week; 4 = drinks 5-7 days per week.

2.4.9.3. Offspring physical activity

Offspring physical activity was rated based on amount of self-reported average physical activity. The following variable was computed: 1 = exercises less than once per week; 2 = exercises 1-2 times per week; 3 = exercises 3-4 times per week; 4 = exercises 5+ times per week.

2.4.9.4. Offspring fast food consumption

Offspring's average consumption of fast food/ready meals was coded as follows: 0 = less than once per week; 1 = 1-2 times per week; 3-4 times per week; 3 = 5-7 times per week; 4 = more than once a day.

2.4.9.5. Offspring medication use

Offspring reported their medication use at the time of the interview as well as on the day of saliva collection. Two variables were constructed one that coded medication use on the day of the interview (relevant to blood collection) and one that updated this with medication use on the day of the saliva sample (relevant to cortisol analyse). Medication with anti-inflammatory effect was coded according to guidance from Danese and colleagues (2007). The following medications were rated as having anti-inflammatory effect: contraceptive pill; asthma pump; hormone regulation medication (e.g. hair regrowth serums); antidepressants; contraceptive injection; non-steroidal anti-inflammatory drugs; corticosteroids; steroid nasal spray; prescription anti-inflammatories.

2.4.10. Offspring physiological measures at 25 years

Salivary cortisol was collected to index offspring's hypothalamic-pituitary-adrenal (HPA) axis function using six Salivette swabs (Sarstedt, Leicester, UK), which were given to participants to take home at the end of the interview.

Offspring's inflammatory biomarkers (high sensitivity C-reactive protein [hsCRP]), and metabolic parameters (lipid levels and glucose levels [glycosylated hemoglobin]) were measured via blood samples collected on the day of the interview. In cases where it was not possible to collect blood on the day of the assessment, samples were collected at a later point in time. In total, 82 (79.6%) participants provided a blood sample. Seventy-three (89.0%) offspring provided a blood sample on the day of the interview. Blood samples were taken from the antecubital fossa using the BD Vacutainer Safety-Lok™ Blood Collection Set (BD, Oxford, UK). The time of blood collection varied, with most (81.7%, *n* = 67) samples being collected between 10:30 and 15:00 hours. Blood was collected from nine (11.0%) participants in the afternoon (15:00-18:00) and from six (7.3%) participants in the evening (18:00-20:30). Participants were not fasted before sample collection. All blood particle measurements were conducted by KCH Phlebotomy Department, who received whole blood samples on the day of the interview, usually within 20 minutes of venepuncture. Analysis took place blind to participant status.

2.4.10.1. Salivary cortisol

The purpose of collecting salivary cortisol was to provide an index of HPA axis function. Participants were instructed to collect four saliva samples in the first hour immediately after awakening ([i] upon awakening; [ii] +15 minutes after awakening; [iii] +30 minutes after awakening; [iv] +60 minutes after awakening) followed by the fifth at midday and the final sample at 20:00 hours. Awakening cortisol was collected in order to evaluate the offspring's stress reactivity in response to the stress of awakening: the cortisol awakening response (CAR).

Furthermore, midday and evening cortisol samples were collected so that the diurnal profile could be characterized. Participants were instructed not to drink, eat, smoke or brush their teeth during the first hour after awakening, nor to eat, drink or smoke in the 30 minutes preceding the collection of midday and evening samples. Offspring were given an instruction sheet and record log to detail the exact times of saliva sample collection (Appendix L). Participants were instructed to collect the samples on the closest possible day to the interview (usually the following weekend) and to keep them in the fridge until posting. On arrival at the laboratory, the samples were frozen at -20°C.

Saliva samples were assayed for cortisol using a standard commercial enzyme linked immuno-sorbent assay (ELISA; Salimetrics, Newmarket, UK) by Dr P Zunszain (Lecturer, Institute of Psychiatry, KCL, UK). Details of the technical protocol and method of calculation are provided in Appendix M. Seventy-five (72.8%) participants returned their saliva samples. Cortisol data was successfully assayed for 74 of the awakening, +15 minutes, +30 minutes, midday and 20:00 hours samples, and for 72 of the +60 minutes samples. Criteria for exclusion from data analysis were as follows: (i) incomplete record sheets returned (n = 4); reported illness on the day of sample collection (n = 1). The mean time of saliva collections were as follows: awakening = 08:31 (SD = 1:16); +15 minutes = 08:47 (SD = 1:16); +30 minutes = 09:01 (SD = 1:18); +60 minutes = 09:31 (SD = 1:16); midday = 12:08 (SD = 0:25); evening = 20:14 (SD = 0:33).

In addition to the computation of continuous variables to record saliva concentrations for each time point, delta change statistics and area under the curve (AUC) calculations were also computed. Delta change statistics were calculated for +15, +30 and +60 minute samples, relevant to awakening levels. The purpose of these calculations was to provide an index of stress reactivity during the first hour after awakening. AUC statistics were calculated for the cortisol awakening response (CAR; awakening, +15, +30, +60 minutes post awakening) and for diurnal cortisol levels (awakening, midday, evening). For CAR AUC levels, two statistics were computed:

(i) AUC with respect to ground (AUC_G); and (ii) AUC with respect to increase (AUC_I). CAR AUC_G estimated total cortisol levels during the first awakening hour, whilst CAR AUC_I provided an estimate of increasing cortisol levels from the awakening level. For diurnal cortisol levels only the AUC_G was calculated. AUC calculations have been shown to be a valid and reliable index or cortisol levels collected at multiple time points and were based on the trapezoid formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

2.4.10.2. Inflammatory markers

Peripheral inflammation was indexed through the measurement of high sensitivity C-reactive protein (hsCRP). CRP is an acute phase protein that is synthesised in the liver which circulates in the plasma. Plasma CRP levels reflect chronic low-grade inflammatory conditions. Measurement using the hsCRP assay has been found to be the most reliable. Details of the technical protocol used for the measurement of hsCRP are provided in Appendix J.

Of the 82 participants who provided a blood sample, hsCRP data was returned from KCH Phlebotomy Department for all. As hsCRP levels greater than 10.0mg/L are indicative of acute inflammation (Danese et al., 2007), this upper limit was used as a cut-off to exclude individuals with potential acute inflammation from all further analysis. Four participants were excluded from analysis on this basis. A continuous variable of offspring's hsCRP levels was generated. In addition, offspring were also dichotomised into low (0) and high (1) inflammation using a cut-off of ≥ 3.0mg/L. This cut-off has been shown to predict high risk of CVD (Ridker, 2003) and is also considered an acceptable cut-off to identify high inflammation amongst the research community (Danese et al., 2007).

2.4.10.3. Metabolic parameters

2.4.10.3.1 Glycosylated haemoglobin

Glycosylated haemoglobin (HbA1c) provides an indirect measure of general glucose levels over the last four months. During the lifespan of the red blood cell (normally 120 days) it is possible for plasma glucose to become attached to the haemoglobin in red blood cells. This process is referred to as glycosylation of haemoglobin. Once a haemoglobin molecule is glycosylated it remains that way. Higher levels of plasma glucose result in increased levels of HbA1c. A build-up of plasma HbA1c reflects the average level of plasma glucose to which the cell was exposed during its lifecycle, thus providing a marker of average blood glucose levels over the preceding months. HbA1c estimates were returned for 98.8% (*n* = 81) of offspring who provided a blood sample. A continuous variable of HbA1c levels was computed. Furthermore, offspring were dichotomized into low (0) and high (1) HbA1C levels using a cut-off of > 6%, in accordance with recommendations from the International Diabetes Foundation (IDF; 2006).

2.4.10.3.2 Full lipid profile

Measures of offspring's full lipid profile included measurement of the following, as present in serum: total cholesterol (CHOL); triglycerides (TGs); high-density lipoprotein cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-C). Measurement of serum CHOL and TG levels provided an estimate of offspring's overall lipid levels. Raised serum CHOL has been associated for many years with an increased risk of atherosclerotic plaque formation leading to an increased likelihood cardiovascular disease (CVD), in particular coronary heart disease (CHD).

TG concentrations (in conjunction with serum CHOL) are useful in the diagnosis and treatment of patients with suspected atherosclerosis, a primary risk factor for CVD.

Measurement of serum HDL-C and LDL-C allowed for an indirect estimate of high- and low-density lipoprotein levels, respectively. High-density lipoprotein (HDL) is a lipoprotein that plays an essential role in CHOL transport and metabolism, principally by transporting excess CHOL from peripheral tissues to the liver. HDL-C measurements enable LDL-C to be calculated, which this is one of the most important lipoproteins in the assessment of CHD. HDL levels are also an independent risk factor for CHD, demonstrating an inverse relationship to disease risk. The Friedwald formula allows for the estimated measurement of LDL particles by subtracting the amount of CHOL associated with other measured particles such as HDLs and TGs (Rifai et al., 1992). Details of the technical protocols used for the calculations of each lipid parameter are provided in Appendix K.

Estimates of CHOL, TGs and HDL-C levels were provided for all offspring who provided a blood sample (*n* = 82), whilst LDL-C data was available for eighty-one (98.8%) participants. Continuous variables for each lipid parameter were generated. In addition, dichotomous variables were also computed to identify offspring with values outside the references ranges for each parameter. Dichotomous variables were classified 0 = normal range, 1 = abnormal range. Cut-offs based on the most conservative reference ranges as set by either the IDF (2006), the World Health Organization (1999), or the European Group for the Study of Insulin Resistance (EGIR; Beck-Nielsen, 1999) were applied. CHOL: > 5.0nmol/L = abnormal. TGs: > 2.0nmol/L = abnormal. HDL-C: < 1.0 = abnormal. LDL-C: > 3.0 nmol/L = abnormal.

2.4.10.3.3 Body mass index and waist circumference

Offspring were weighed using an electronic weighing scale calibrated to the nearest .05kg. Offspring were weighed wearing light clothing and without shoes. Height was measured without shoes to the nearest 0.5 cm using a measuring tape. Waist circumference (WC) was measured to the nearest .5 cm using a measuring tape. Participants were asked to stand straight, exhale, and

relax. WC was measured in line with the naval. The body mass index (BMI) provides a statistical measure of a person's weight scaled according to height. BMI was calculated as the individual's body weight (in kilograms) divided by the square of their height (in metres). Height, weight and WC measurements were collected from 102 (99.0%) participants. Offspring with BMIs ≥ 30.0 kg/m^2 were classified as obese.

2.4.10.3.4 Cumulative risks index

A cumulative risks index for metabolic syndrome was constructed by summing the number of metabolic parameters for which an offspring fell outside the normal range (based on dichotomized parameter variables). The following parameters were included in the risk index: (i) high HbA1c; (ii) high CHOL; (iii) high TGs; (iv) low HDL-C; (v) high LDL-C; (v) obese BMI. A cumulative risks index is consistent with the idea that: (i) although the effect on one parameter might be weak, the effects on multiple parameters can be quite large; and (ii) because parameter risks tend to cluster together, the number of abnormal parameters, rather than a particular parameter, will index greater disturbance in the metabolic system.

2.5. Data analysis

All statistical analyses were conducted in IBM SPSS Statistics Version 21 (IBM Ltd., Portsmouth, UK). Data was assessed for normality using probability-probability plots and the Kolmogorov-Smirnov test, and for homogeneity of variance using Levene's test. Log and square root transformations were applied to improve the normality and homogeneity of the data. For data that did not benefit from transformation, non-parametric statistical tests were applied.

Descriptive statistics are presented as mean (*M*) and standard deviation (*SD*), or frequency and percentage (%), where appropriate. In graphs, standard error of the mean (*SE*) is presented. For univariate analyses, the independent samples *t*-test was used for group comparisons comprising continuous parametric data, whilst the Mann-Whitney test was applied to non-parametric continuous data, reported by *z* score. Pearson's chi square (χ^2) test for independence was used for the analysis of categorical data. Fisher's exact test was applied in cases where one contingency cell yielded an expected count of less than five. Pearson's correlation (*r*) was used for the analysis of association between two parametric continuous variables, whilst Spearman's correlation (r_s) was applied to continuous non-parametric data. The point-biserial correlation (r_{pb}) was applied to test for association between a continuous parametric variable and dichotomous variable, whilst the r_{pb} was applied to raked scores for non-parametric data. Kappa (κ) statistics are reported for inter-associations between two dichotomous variables.

Hierarchical multiple logistic regressions were applied to data with dichotomous outcome variables; whilst hierarchical multiple linear regressions were used for data with continuous outcome variables. In call cases, the blockwise (hierarchical) method of entry was used, with the variable of interest entered at the final step. Tests for multicollinerarity were conducted for all multiple regression analyses. When ANOVAs were used, Gabriel's test was applied in *post hoc* analyses of pairwise comparisons. Gabriel's test adjusts for multiple testing as well as for

unequal group sizes, and is designed to cope with situations in which small sample sizes are different.

As a general rule, statistics are reported to one decimal place except where two decimal places would yield particularly informative further information, and when reporting p values. *P* values > .05 are reported to two decimal places, whilst *p* values < .05 are reported to three. In tables, all significant results are highlighted in **bold** or with the use of an asterisk (*).

2.5.1. Mediation analysis

Mediation analysis is a statistical method used to help answer the question of how some causal agent, X, transmits its effect on Y. The most basic of mediation models – the simple mediation model – is represented in conceptual form in Figure 3. As can be seen, the model contains two consequent variables, Y and M, and two antecedent variables, X and M. In such a model, X is proposed to influence outcome Y though M. There are two pathways linking X to Y. One pathway leads from X to Y without passing through M, the *direct effect* (path *c'*), and a second passes from X to M (path a) and then from M to Y controlling for X (path *b*). This latter pathway is known as the *indirect effect* (path *ab*), and is quantified by computing the product term of the coefficients *a* multiplied by *b* (*ab*). Quantitatively, the *indirect effect* (*ab*) and *direct effect* (*c'*) sum to make the *total effect* (*c*) of X on Y. This nomenclature has become customary in mediation methodology (Hayes, 2013).

When empirically testing a causal process that involves mediation, it is necessary to estimate the size of the direct and indirect effects, along with conducting inferential tests of their values (i.e. are they significantly different from zero). Estimates of these effects can be calculated simply by using ordinary least square regression (OLS) techniques to estimate the individual paths (e.g. path *b* = coefficient of Y regressed on M controlling for X).

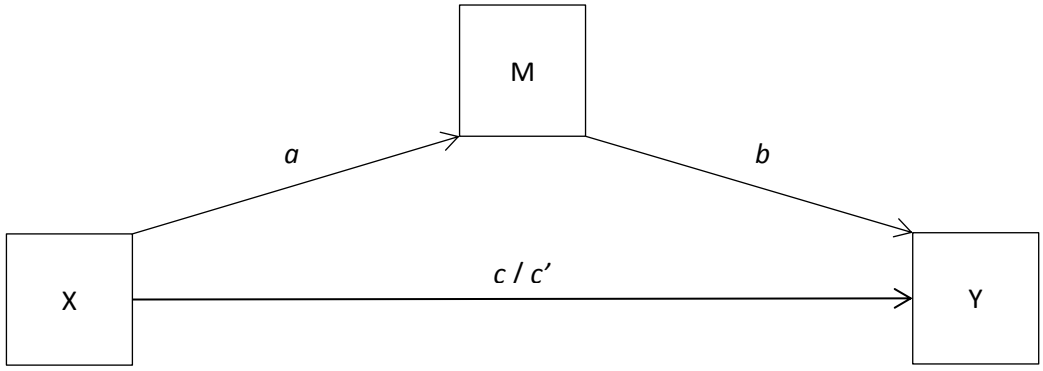

Figure 3. Conceptual diagram of a simple mediation model

In classic mediation techniques, such as the causal steps approach, as popularised by Baron and Kenny (1986), there is no formal quantification of the indirect effect. Rather, support for the fact that M functions as a mediatory between X and Y focuses on the outcomes of a series of tests of significance for each path in the causal system. First, one must establish that X and Y (path *c*) are associated by regressing Y on X. Assuming this criterion is met, evidence that X affects M (path *a*) is sought through regressing M on X. Third, a test of whether M affects Y controlling for X (path *b*) is undertaken by regressing Y on both X and M. If the null hypothesis that *b* equals zero cannot be rejected, then the procedure stops. If however, the null hypothesis can be rejected, then the size of the total effect (path *c*) is compared to the size of the direct effect (path *c'*; Y regressed on X controlling for M). If *c'* is closer to zero than *c* and *c'* is not statistically significant, then it is claimed that M *completely* mediates X's effect on Y. By contrast, if *c'* is closer to zero than *c* but *c'* is statistically significant then is it claimed that M *partially* mediates X's effect on Y. Whilst this methodology is popular, it has many problems. Chiefly, there is no formal quantification of the indirect effect; rather the existence of an indirect effect is inferred using logic about the set of outcomes from tests about the quantification of something other than the indirect effect. Furthermore, this assertion rests upon the rebuttal of three null hypotheses, which inflates type II error and consequently reduces power.

The alternative is the formal quantification of the indirect effect (*ab*) followed by an inferential test that it does not equal zero. There are numerous approaches to statistical inference of the indirect effect (see MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002; Preacher & Hayes, 2008). However one of the most robust methods is the use of bootstrap confidence intervals (Hayes, 2013). This technique is superior as it does not make any assumptions about the nature of the distribution of the indirect effect, thus it is a non-parametric statistical method. Bootstrapping is the process of generating a population estimate of the statistic of interest by treating the collected sample as a miniature representation of the population. Observations in the sample are *resampled with replacement*, and the statistic of interest is calculated in the new

sample of size *n* that was constructed through the resampling process. To perform an inference test for the size of the indirect effect (*ab*), bootstrap confidence intervals (CIs) of *ab* are generated through this resampling procedure:

1) create a *bootstrap sample* of *n* cases by randomly sampling cases *with replacement* from the original dataset;
2) for each bootstrap sample estimate the indirect effect *ab*;
3) repeat steps 1 and 2 a total of *j* times, with *j* being at least 1,000 but not more than 10,000;
4) sort the bootstrap estimates from low to high;
5) generate the desired upper and lower CIs of the estimate (of *ab*) by selecting the estimates that define the percentiles of interest (e.g. 2.5^{th} and 97.5^{th} percentiles for $\alpha = .05$).

Once this process is completed, the (percentile) bootstrap CIs of *ab* are then used to signify whether *ab* is different from zero. If the CIs include zero the null hypothesis cannot be rejected, however if they do not contain zero, the null hypothesis can be rejected with *q* confidence, where *q* is the s percentile level selected in step 4. A variation of this method is the generation of *biased-corrected* bootstrap CIs. These are very similar to percentile bootstrap CIs, but have the endpoints adjusted according to the proportion of *j* estimates of *ab* that are less than the original *ab* estimate. These endpoints are adjusted upwards or downwards to varying degrees depending on the proportion, thus giving a more valid CI estimate.

Throughout this thesis, the generation of 95% bias-corrected bootstrap CIs are generated as an inferential test of the indirect effect in mediation analyses. All statistical analyses were conducted using PROCESS for SPSS Version 2.041 (Prof A Hayes, The Ohio State University, USA).

CHAPTER 3: RESULTS

3.1. Part 1: Depression in young adulthood

3.1.1. Overview

The primary goal of the first set of analyses was to test the hypothesis that offspring exposure to maternal depression during gestation represents a very early life environmental insult that elevates risk for depressive psychopathology during young adulthood. The secondary goal was to test whether child maltreatment moderates this association. First, descriptive statistics are provided for the sample at 25 years. Next, I investigate the interrelationships between risk factors spanning the gestational period through to young adulthood. Regression analyses are then applied in order to quantify the impact of prenatal risk factors for offspring depression. Next, possible psychosocial pathways from offspring exposure to depression *in utero* to adulthood depression are explored using moderation and mediation analyses. Finally, to further quantify these pathways, ANOVAs are performed.

3.1.2. Descriptive analyses

3.1.2.1. Sample characteristics at 25 years

One hundred and three offspring participated in the 25-year follow-up. Basic offspring sociodemographic characteristics are presented in Table 3. The majority (72.8%) of offspring were of white British origin. There was a comparable ratio of males (47.6%) to females (52.4%). Less than half (45.6%) of the sample had pursued higher education at 25 years. 8.7% of offspring had married, whilst a large proportion (39.8%) still lived in the family home. Almost a fifth of offspring (18.5%) were unemployed. Over a third (36.9%) had fathered/borne at least one child.

Table 3. Offspring sociodemographic characteristics at 25 years

Characteristic	Statistic
Age, *M* (*SD*)	25.1 (.7)
Gender, % female	52.4
Ethnicity, %	
White British	72.8
White non-British	3.9
Black Caribbean	8.7
Black African	3.9
Asian Indian/Pakistani/Sri Lankan	2.0
Oriental Chinese/Vietnamese/Filipino	2.9
Mixed	5.8
Highest level of education, %	
No qualifications	10.7
GCSEs	4.9
NVQs, levels 1-3	35.9
A Levels	2.9
HND	5.8
Bachelor degree	29.1
Postgraduate qualification	10.7
Marital status, %	
Single	34.0
Partner, not co-habiting	25.3
Partner, co-habiting	32.0
Married	8.7
Housing, %	
Homeowner	7.8
Privately rented accommodation	33.0
Accommodation rented from housing association	9.7
Council housing	8.7
Parental home	39.8
Her Majesty's Prison	1.0
Employment status, %	
Part-time employment	12.6
Full-time employment	60.2
Full-time student	8.7
Unemployed	18.5
Borne/fathered a child, %	36.9

3.1.2.2. Adult offspring DSM-IV depression

DSM-IV diagnoses of offspring depressive disorders through young adulthood (18-25 years) were rated at 25 years in order to capture instances of clinically relevant emotional psychopathology. Thirty-nine (37.9%) offspring met DSM-IV criteria for a diagnosis of depression (major depressive disorder [MDD], dysthymic disorder, depressive disorder not otherwise specified [NOS]) between the ages of 18 and 25. Twenty-nine (28.2%) offspring had experienced at least one MDD episode, five (4.9%) offspring experienced dysthymia and five (4.9%) offspring met criterion for a diagnosis of depressive disorder NOS. The mean number of DSM-IV depressive symptoms throughout this period was 2.3, *SD* = 3.1. Eleven (10.7%) offspring were currently depressed at the time of the interview.

3.1.2.3. Maternal depression from pregnancy to 16 years

3.1.2.3.1 Perinatal period

Thirty-five (34.0%) of the offspring's mothers had experienced a depressive episode during pregnancy (20 and/or 36 weeks gestation). Twenty-six (25.2%) mothers were depressed at 3 months postpartum, and 19 (18.8%) mothers were depressed at 1 year postpartum. Thus, there was a pattern of decreasing rates of maternal depression through the perinatal period into offspring infancy. There was a high degree of association between prenatal and postnatal depression. Of the 35 mothers depressed during pregnancy, 16 (45.7%) were depressed at 3 months postpartum, $\chi^2(1) = 11.8$, $p = .001$, *OR* = 4.9, 95% CI [1.9, 12.6], whilst a total of 20 (57.1%) mothers went on to experience depression again at some point during the first postnatal year, $\chi^2(1) = 11.5$, $p = .001$, *OR* = 4.3, 95% CI [1.8, 10.4].

3.1.2.3.2 Childhood

The prevalence of maternal depression across the offspring's childhood years was calculated. Thirty-nine (38.2%) mothers were depressed at some point during the offspring's early childhood (1-4 years), 22 (21.8%) mothers were depressed at some point during the offspring's middle to childhood (4-11 years) and 29 (29.9%) mothers were depressed at some point during the offspring's late childhood (11-16 years). Overall, 64 (62.7%) mothers experienced at least one depressive episode between the offspring's birth and 16 years.

There was a high degree of association between maternal depression during pregnancy and maternal depression through childhood (birth to 16). The majority (88.6%, n = 31) of mothers who were depressed prenatally went on to experience another episode of depression at some point between the child's birth and 16th birthday, $\chi^2(1) = 15.2$, $p < .001$, $OR = 8.0$, 95% CI [2.5, 25.1].

3.1.2.4. Offspring childhood maltreatment

Offspring experience of severe abuse and neglect up to age 17 was rated in order to ascertain a measure of childhood maltreatment. Twenty (19.4%) offspring experienced physical abuse, 11 (10.7%) offspring experienced sexual abuse, 17 (16.5%) offspring experienced parental emotional abuse and 15 (14.6%) offspring experienced parental neglect. The distribution of these prevalence rates across offspring were as follows: 23 (22.3%) offspring experienced one form of maltreatment, 3 (2.9%) offspring experienced two forms of maltreatment, six (5.8%) offspring experienced three types of maltreatment and four (3.9%) offspring experienced all forms of maltreatment. The inter-associations between types of maltreatment are presented in Table 4. Given that maltreatment was rated if an offspring experienced one or more forms of abuse and neglect, overall, 36 (35.0%) offspring were classed as having been maltreated in childhood.

Table 4. Inter-associations between types of maltreatment

	1	**2**	**3**	**4**
(1) Physical abuse	-			
(2) Sexual abuse	.14	-		
(3) Emotional abuse	.38***	.43***	-	
(4) Neglect	.28**	.47***	.63***	-

Note. Kappa coefficients are presented.
** $p < .01$, *** $p < .001$.

3.1.2.4.1 Perpetrators of offspring physical and sexual abuse

Parents were observed to be perpetrators for the majority (90.0%, *n* = 18) of cases of physical abuse, but were not perpetrators to any instances of sexual abuse. Non-family members accounted for the largest proportion of perpetrators of sexual abuse (63.6%, *n* = 7). Frequency statistics for all perpetrator groups across all instances of physical and sexual abuse are presented in Table 5.

3.1.2.4.2 Perpetrators of offspring emotional abuse and neglect

Fathers were found to account for the majority of perpetrators of parental emotional abuse (64.7%, *n* = 11) and neglect (73.3%, *n* = 11). Rates of emotional abuse and neglect by perpetrators are presented in Table 6.

Table 5. Perpetrators of offspring physical and sexual abuse

	Offspring abuse	
	Physical abuse, % (*n*)	**Sexual abuse, % (*n*)**
Perpetrator	***n* = 20**	***n* = 11**
Parent	90.0 (18)	–
Stepparent	5.0 (1)	9.0 (1)
Other family member	15.0 (3)	36.3 (4)
Non-family member	–	63.6 (7)

Note. Frequency statistics index prevalence rates for each perpetrator class. Where offspring experienced abuse involving multiple perpetrators, all perpetrators are reported separately.

Table 6. Perpetrators of parental emotional abuse and neglect

	Offspring parental emotional abuse and neglect	
	Emotional abuse, % (*n*)	**Neglect, % (*n*)**
Perpetrator	***n* = 17**	***n* = 15**
Mother only	35.3 (6)	26.7 (4)
Father only	47.1 (8)	66.7 (10)
Both parents	17.6 (3)	6.6 (1)

3.1.2.4.3 Agreement between prospective and retrospective reporting of abuse

Whilst offspring experience of emotional abuse and neglect was rated at 25 years through retrospective report, reports of physical and sexual abuse were provided both prospectively during the offspring's child and adolescent years, as well as retrospectively at 25 years. At 11 and 16 years both offspring and parents provided interview reports about any lifetime incidents of sexual and physical abuse, and at 25 years offspring provided interview reports about any incidents of sexual and physical abuse occurring since the start of memory up until age 17.

Of the 20 offspring rated as having experienced physical abuse, reports for 7 (35.0%) cases were provided only during childhood, reports for 8 (40.0%) cases were provided only in adulthood, whilst reports for 5 (25.0%) of the cases were provided in both childhood and adulthood. This translates into a kappa of .32, $p = .001$. Of the 11 offspring who experienced sexual abuse, reports for 3 (27.3%) cases were provided in only childhood, reports for a further 3 cases (27.3%) were provided in only adulthood, and reports for 5 (45.4%) cases were provided in both childhood and adulthood. Thus, agreement between prospective and retrospective reports of sexual abuse was moderate, $\kappa = .59$, $p < .001$.

3.1.3. Does prenatal maternal depression predict offspring depression in young adulthood?

The first step to testing the first hypothesis that prenatal maternal depression predicts offspring depression in young adulthood, involved comparing offspring whose mothers had suffered depression during pregnancy and those whose mothers had not, on their (offspring) experience of clinically relevant depressive psychopathology across the entire young adulthood period (18-25 years). I performed analyses on both diagnoses of depression and symptom levels. Categorical between-group comparisons were performed using diagnostic status. Further, more fine-grained analyses considered symptom levels.

3.1.3.1. Association between prenatal maternal depression and offspring adulthood depression

There was a significant association between prenatal maternal depression and offspring depressive disorders in young adulthood, $\chi^2(1) = 8.4$, $p = .004$, $OR = 3.4$, 95% CI [1.5, 8.1]. Of the 35 offspring exposed to maternal depression *in utero*, 20 (57.1%) were rated with a depression diagnosis in young adulthood. In contrast, of the 68 offspring not exposed to maternal prenatal depression, only 19 (27.9%) were rated with a depression diagnosis in young adulthood. Similarly, the mean number of adult offspring DSM-IV depressive disorder symptoms was significantly greater, $M = 3.4$, $SD = 3.2$, amongst offspring exposed to maternal depression *in utero*, compared to non-prenatally exposed offspring, $M = 1.7$, $SD = 2.8$, $z = -2.8$, $p = .004$.

3.1.3.2. Group differences between offspring exposed to maternal depression *in utero* and non-exposed offspring

In order to examine the interrelationships between exposure to prenatal maternal depression and further adversity factors, offspring exposed to maternal depression *in utero* were compared

to non-exposed offspring on all further potential risk variables. Data for these analyses are presented in Table 7.

To summarise, mothers who were depressed during pregnancy did not differ on any sociodemographic characteristics compared to mothers who were not depressed during pregnancy (see Table 7). However, mothers who were prenatally depressed did report significantly more anxiety symptoms during pregnancy, M = 6.6, SD = 3.3, compared to non-depressed mothers, M = 4.2, SD = 3.0; z = -3.6, $p < .001$. Whether an offspring had or had not been exposed to maternal depression *in utero*, was not associated with any of their (offspring) sociodemographic characteristics. In contrast, offspring exposure to prenatal maternal depression was significantly associated with further exposure to maternal depression across all developmental periods from birth to 16 years (see Table 7). Furthermore, the prevalence of childhood maltreatment was significantly higher amongst offspring exposed to maternal depression *in utero* (48.6%), compared to non-exposed offspring, 27.9%, $\chi^2(1) = 4.3$, $p = .038$, *OR* = 2.4, 95% CI [1.0, 5.7]. This is a replication, using newly collected data, of the findings published by Pawlby and colleagues (2011) that were based on ratings of offspring experiences of physical abuse, sexual abuse and harsh discipline at 11 years (see 1.7.2)

Table 7. Group differences between offspring exposed to prenatal maternal depression compared to non-exposed offspring

	Exposure to maternal depression *in utero*		
	None	**Exposed**	
	***n* = 68**	***n* = 35**	**Group effect**
Offspring adulthood psychopathology			
Depressive disorder diagnosis, %	27.9	57.1	**$\chi^2(1) = 8.4$, $p = .004$**
Depressive disorder symptoms, *M* (*SD*)	1.7 (2.8)	3.4 (3.2)	**$z = -2.8$, $p = .004$**
Maternal characteristics			
Age at index pregnancy, *M* (*SD*)	26.4 (4.6)	26.0 (5.3)	$t(101) = .4$, $p = .68$
Ethnicity, %			
White British	77.9	77.1	$\chi^2(1) = .01$, $p = .93$
Not white British	22.1	22.9	
Social class, %			
Middle class	13.2	11.4	$\chi^2(1) = .1$, $p > .99$ [a]
Working class	86.8	88.6	
Education, % some qualifications	77.9	71.4	$\chi^2(1) = .5$, $p = .47$
Marital status at pregnancy, %			
Married	69.1	57.1	$\chi^2(1) = 1.5$, $p = .23$
Not married	30.9	42.9	
Previous psychiatric history, % [b]	23.9	37.1	$\chi^2(1) = 2.0$, $p = .16$
Maternal perinatal factors			
Anxiety in pregnancy, *M* (*SD*)	4.2 (3.0)	6.6 (3.3)	**$z = -3.6$, $p < .001$**
Medical problems in pregnancy, *%* [c]	28.1	44.1	$\chi^2(1) = 2.5$, $p = .11$
Prenatal smoking (cigarettes/day), *M* (*SD*)	3.4 (6.1)	5.0 (6.3)	$z = -1.3$, $p = .18$
Prenatal drinking (drinks/week), *M* (*SD*)	1.1 (2.0)	.6 (2.0)	$z = -.9$, $p = .36$
No. weeks breastfed, *M* (*SD*) [d]	8.6 (9.2)	6.8 (9.0)	$z = -1.2$, $p = .23$
Postnatal depression, %			
During 1st postnatal year	23.5	57.1	**$\chi^2(1) = 11.5$, $p = .001$**
3 months	14.7	45.7	**$\chi^2(1) = 11.8$, $p = .001$**
12 months [e]	10.4	35.3	**$\chi^2(1) = 9.1$, $p = .003$**
Offspring obstetric outcomes			
Birth weight, *M* (*SD*)	3387.3 (490.4)	3373.3 (502.4)	$t(101) = .1$, $p = .89$
Gestational age, *M* (*SD*)	39.9 (1.7)	39.7 (1.8)	$z = -.8$, $p = .44$
Childhood factors			
Maternal depression 1 to 16 years, %			
1-16 [b]	49.3	88.6	**$\chi^2(1) = 15.2$, $p < .001$**
1-4 [b]	29.9	48.7	**$\chi^2(1) = 5.8$, $p = .016$**
4 -11 [f]	10.6	42.9	**$\chi^2(1) = 14.0$, $p < .001$**
11-16 [g]	20.6	47.1	**$\chi^2(1) = 7.4$, $p = .007$**
Offspring childhood maltreatment, %			
Maltreatment composite	27.9	48.6	**$\chi^2(1) = 4.3$, $p = .038$**
Physical abuse	11.8	34.3	**$\chi^2(1) = 7.5$, $p = .006$**

(continued)

	Exposure to maternal depression *in utero*		
	None	**Exposed**	
	***n* = 68**	***n* = 35**	**Group effect**
Sexual abuse	5.9	20.0	**$\chi^2(1) = 4.8$, $p = .028$** [a]
Emotional abuse	10.3	28.6	**$\chi^2(1) = 5.6$, $p = .018$**
Neglect	10.3	22.9	**$\chi^2(1) = 2.9$, $p = .09$**
Offspring characteristics			
Gender, % female	50.0	57.1	$\chi^2(1) = .5$, $p = .49$
Ethnicity, %			
White British	75.0	68.6	$\chi^2(1) = .5$, $p = .49$
Not white British	25.0	31.4	
Education, % some qualifications	91.2	85.7	$\chi^2(1) = .7$, $p = .50$ [a]
IQ, *M* (*SD*) [h]	96.3 (15.1)	93.0 (15.5)	$t(97) = 1.0$, $p = .31$

Note. Group effects are based upon (i) Pearson's χ^2 test for independence for associations with two dichotomous variables, (ii) the independent samples *t*-test for associations with a dichotomous and continuous variable that permitted parametric analyses, and (iii) the Mann-Whitney test for associations with a dichotomous and continuous variable that did not permit parametric analyses. [a] Fisher's exact test applied due to one contingency table cell yielding an expected cell count less than five; [b] *n* = 102 (non-exposed: 67, exposed: 35); [c] *n* = 98 (64, 34); [d] *n* = 59 (37, 22); [e] *n* = 101 (67, 34); [f] *n* = 101 (66, 35); [g] *n* = 97 (63, 34); [h] *n* = 99 (65, 34).

3.1.3.3. Univariate analyses of further potential risk factors for offspring adulthood depression

In order to identify additional risk factors for offspring adulthood depression, depressed versus non-depressed offspring were compared on all potential risk variables. Results for the analyses performed using depressive disorder diagnoses are presented in Table 8, whilst results for depressive symptoms are presented in Table 9. I also conducted univariate analyses of the inter-associations between risk variables, the results for which are presented in Table 10.

The pattern of findings was highly similar between offspring diagnoses and symptoms. Amongst sociodemographic characteristics, maternal marital status was significantly associated with adult offspring depressive disorders, $\chi^2(1) = 5.2$, $p = .022$, $OR = 2.6$, 95% CI [1.1, 25.1], and symptoms, $r_{pb} = .24$, $p = .013$. Significantly more mothers of depressed offspring were unmarried in pregnancy (48.7%) compared to mothers of non-depressed offspring (28.6%). There was a trend ($p < .10$) for more female offspring to have a depressive disorder diagnosis. This association (between gender and adult offspring depressive symptoms) was found to be statistically significant for symptom levels, $r_{pb} = .20$, $p = .048$.

In terms of childhood factors, offspring exposure to maternal depression during early childhood (1-4 years) and late childhood (11-16 years) were both associated significantly with adult offspring depressive disorder diagnoses, 1-4 years: $n = 102$, $\chi^2(1) = 6.5$, $p = .011$, $OR = 2.9$, 95% CI [1.3, 2.7]; 11-16 years: $n = 97$, $\chi^2(1) = 11.0$, $p = .001$, $OR = 4.5$, 95% CI [1.8, 11.4], and symptoms, 1-4 years: $n = 102$, $r_{pb} = .25$, $p = .011$; 11-16 years: $n = 97$, $r_{pb} = .31$, $p = .002$. There was also a positive association between offspring exposure to childhood maltreatment and elevated rates of offspring adulthood depressive disorders, $\chi^2(1) = 5.2$, $p = .022$, $OR = 2.6$, 95% CI [1.1, 6.1], and symptoms, $r_{pb} = .26$, $p = .009$.

Table 8. Group differences between depressed versus non-depressed adult offspring

	Adult offspring		
	Well	Depressed	
	***n* = 64**	***n* = 39**	**Group effect**
Maternal characteristics			
Age at index pregnancy, *M* (*SD*)	26.5 (4.8)	25.8 (4.9)	$t(101) = .7$, $p = .47$
Ethnicity, %			
White British	79.7	74.4	$\chi^2(1) = .4$, $p = .53$
Not white British	20.3	25.6	
Social class, %			
Middle class	17.2	5.1	$\chi^2(1) = 3.2$, $p = .12$ [a]
Working class	82.8	94.9	
Education, % some qualifications	79.9	69.2	$\chi^2(1) = 1.4$, $p = .23$
Marital status in pregnancy, %			
Married	73.4	51.3	**$\chi^2(1) = 5.2$, $p = .022$**
Not married	26.6	48.7	
Previous psychiatric history, % [b]	25.4	33.3	$\chi^2(1) = .7$, $p = .39$
Maternal perinatal factors			
Depression in pregnancy, %	23.4	51.3	**$\chi^2(1) = 8.4$, $p = .004$**
Anxiety in pregnancy, *M* (*SD*)	4.7 (3.1)	5.6 (3.6)	$z = -1.2$, $p = .25$
Medical problems in pregnancy, *%* [c]	38.1	25.7	$\chi^2(1) = 1.5$, $p = .21$
Prenatal smoking (cigarettes/day), *M* (*SD*)	3.7 (5.8)	4.1 (6.8)	$z = -.1$, $p = .90$
Prenatal drinking (drinks/week), *M* (*SD*)	.8 (1.5)	1.1 (2.0)	$z = -1.3$, $p = .19$
Breastfeeding (weeks), *M* (*SD*) [d]	7.8 (8.3)	8.3 (10.4)	$z = -.7$, $p = .46$
Postnatal depression, %			
1st postnatal year	29.7	43.6	$\chi^2(1) = 2.1$, $p = .15$
3 months	18.8	35.9	$\chi^2(1) = 3.8$, $p = .052$
12 months [e]	19.4	17.9	$\chi^2(1) = .03$, $p = .86$
Offspring obstetric outcomes			
Birth weight, *M* (*SD*)	3399.1 (432.3)	3355.4 (582.3)	$t(101) = .4$, $p = .66$
Gestational age, *M* (*SD*)	40.1	39.6	$z = -1.1$, $p = .27$
Childhood factors			
Maternal depression 1 to 16 years, %			
1-16 [b]	52.4	79.5	**$\chi^2(1) = 7.6$, $p = .006$**
1-4 [b]	28.6	53.8	**$\chi^2(1) = 6.5$, $p = .011$**
4-11 [f]	19.4	25.6	$\chi^2(1) = .56$, $p = .46$
11-16 [h]	18.0	50.0	**$\chi^2(1) = 11.0$, $p = .001$**
Offspring childhood maltreatment, %			
Maltreatment composite	26.6	48.7	**$\chi^2(1) = 5.2$, $p = .022$**
Physical abuse	14.1	28.2	$\chi^2(1) = 3.1$, $p = .08$
Sexual abuse	3.1	23.1	**$\chi^2(1) = 10.1$, $p = .002$** [a]
Emotional abuse	9.4	28.2	**$\chi^2(1) = 6.2$, $p = .013$**
Neglect	9.4	23.1	$\chi^2(1) = 3.7$, $p = .06$
Offspring characteristics			
Gender, % female	45.3	64.1	$\chi^2(1) = 3.4$, $p = .06$

(continued)

	Adult offspring		
	Well	Depressed	
	***n* = 64**	***n* = 39**	**Group effect**
Ethnicity, %			
White British	76.9	66.7	$\chi^2(1) = 1.2$, $p = .27$
Not white British	23.4	33.3	
Education, % some qualifications	92.2	84.6	$\chi^2(1) = 1.5$, $p = .33$ [a]
IQ, *M* (*SD*) [h]	95.8	94.1	$t(97) = .5$, $p = .56$
	(16.1)	(13.8)	

Note. Group effects were based upon (i) the Pearson's χ^2 test for independence for associations with two dichotomous variables, (ii) the independent samples *t*-test for associations with a dichotomous and continuous variable that permitted parametric analyses, and (iii) the Mann-Whitney test for associations with a dichotomous and continuous variable that did not permit parametric analyses.

[a] Fisher's exact test applied due to one contingency table cell showing an expected cell count less than five; [b] *n* = 102 (well: 63, depressed: 39); [c] *n* = 98 (63, 35); [d] *n* = 59 (37, 22); [e] *n* = 101 (62, 39); [f] *n* = 101 (62, 39); [g] *n* = 97 (61, 36); [h] *n* = 98 (61, 37).

Table 9. Correlations between adult offspring depressive disorder symptoms and risk variables

	Adult offspring DSM-IV depressive symptoms	
	Coeff.	***p* value**
Maternal characteristics		
Age at index pregnancy	-.10	.33
Ethnicity (white British vs. not white British)	.04	.68
Social class (middle class vs. working class)	.19	.06
Education (some vs. no qualifications)	.12	.25
Marital status at pregnancy (married vs. unmarried)	.24	**.013**
Previous psychiatric history [a]	.06	.54
Maternal perinatal factors		
Prenatal depression	.28	**.004**
Prenatal anxiety	.08	.41
Prenatal medical problems [b]	-.12	.23
Prenatal smoking (cigarettes/day)	-.03	.74
Prenatal drinking (drinks/week)	.16	.12
No. weeks breastfed [c]	-.08	.54
Postnatal depression		
1st postnatal year	.11	.28
3 months	.16	.10
12 months [d]	-.03	.75
Offspring obstetric outcomes		
Birth weight	-.05	.60
Gestational age	-.12	.22
Childhood factors		
Maternal depression 1 to 16		
1-16 [a]	.25	**.010**
1-4 [a]	.25	**.011**
4-11 [d]	.11	.27
11-16 [e]	.31	**.002**
		(continued)
Offspring childhood maltreatment		
Maltreatment composite	.26	**.009**
Physical abuse	.18	.07
Sexual abuse	.35	**< .001**
Emotional abuse	.24	**.013**
Neglect	.22	**.029**
Offspring characteristics		
Gender (male vs. female)	.20	**.048**
Ethnicity (white British vs. not white British)	.08	.45
Education (some vs. no qualifications)	.04	.66
IQ [f]	-.07	.48

Note: Spearman's correlation coefficients are reported for continuous predictor variables, whilst point-biserial correlation coefficients based on ranked scores are reported for dichotomous predictors.
[a] n = 102; [b] n = 98; [c] n = 59; [d] n = 101; [e] n = 97; [f] n = 99.

Table 10. Inter-associations between risk variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	*n*
Maternal characteristics																					
(1) Age at preg.	–																				103
(2) Ethnicity	.18†	–																			103
(3) Social class	-.19†	-.09*	–																		103
(4) Education	.06	-.14	.09*	–																	103
(5) Marital status	-.24*	.19*	-.05	-.03	–																103
(6) Psychiatric hx.	.18†	.13	-.01	-.11	.18†	–															102
Maternal perinatal factors																					
(7) Preg. dep.	-.04	.01	.01	.07	.12	.14	–														103
(8) Preg. anxiety	.27**	.06	.02	.12	-.02	.11	.36***	–													103
(9) Preg. med. prob.	.13	.05	.01	.01	.03	.15	-.16	.26**	–												98
(10) Preg. smoking	-.10	-.13	.07	.31**	.37***	-.08	.13	.06	.14	–											103
(11) Preg. drinking	.27**	-.04	-.19†	-.07	.12	-.03	-.09	-.02	.05	-.06	–										103
(12) Breastfeeding	.17	.32*	-.33*	-.18	-.01	-.10	-.16	-.13	-.18†	-.31†	.20	–									59
(13) Dep. birth to 1	.05	-.02	.05	.20*	.10	.39***	.33**	.21*	-.08	.14	-.08	-.06	–								103
Offspring obstetric outcomes																					
(14) Birth weight	.08	-.25*	.01	.03	-.38***	-.31**	-.01	.02	-.18	-.34***	-.02	-.01	-.15	–							103
(15) Gest. age	-.02	-.15	.03	.02	-.36**	-.28**	-.08	.14	-.21*	-.18†	.06	.03	-.04	.48***	–						103
Childhood factors																					
(16) Mat. dep. 1-16	.05	-.02	.15†	.05	.15†	.38***	.33***	.33**	.10	.11	.06	-.22	.50***	-.11	-.09	–					102
(17) Maltreatment	-.25*	.05	.08	.06	.10	.17†	.21*	.01	.07	.10	-.11	.07	.20†	-.19†	-.12	.16†	–				103
Offspring characteristics																					
(18) Gender	-.20*	.07	.03	-.19*	.20*	.10	.06	.01	-.03	.05	.03	-.11	.04	-.22*	-.06	.04	.01	–			103
(19) Ethnicity	.13	.49***	-.10	-.12†	.14†	.10	.05	.09	.08	-.16	-.08	.11	.07	-.17†	-.16	.02	.11	.05	–		103
(20) Education	-.16	-.10	.03	.15†	.06	-.05	.07	-.04	.07	.18†	.02	-.25†	.01	-.01	-.06	-.01	.16*	-.01	.06	–	103
(21) IQ	.03	.14	-.26*	-.20†	.09	-.08	-.10	-.01	.08	-.14	.04	.47***	.30**	.11	.08	-.12	-.09	.05	-.12	-.26*	99

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable (based on rank scores for continuous variables not permitting parametric analysis), whilst kappa coefficients are presented for associations between two dichotomous variables. Preg. = pregnancy; hx. = history; dep = depression; med. = medical; prob. = problems; gest. = gestational; mat. = maternal. (2) white British vs. non-white British; (3) middle class vs. working class; (4) some qualifications vs. no qualifications; (5) married vs. unmarried; (10) cigarettes/day; (11) drinks/week; (18) boy vs. girl; (19) white British vs. non-white British; (20) some qualifications vs. no qualifications. $^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

3.1.3.4. Multiple regression analyses of adult offspring depression

Hierarchical multiple logistic regression analyses were conducted to assess whether mothers' depression during pregnancy explained the variance in offspring depressive disorder diagnoses in young adulthood. The analyses proceeded in three steps. At the first step, sociodemographic characteristics that were found to be associated significantly with offspring depressive disorder diagnoses (namely, maternal marital status) were entered into the model. At the second step, prenatal maternal depression was entered. At this step, prenatal depression was found to predict significantly adult offspring depressive disorders, Wald statistic = 7.0, p = .008, OR = 3.3, 95% CI [1.4, 7.8], model $\chi^2(2) = 12.4$, p = .002. At the final step, further childhood risk variables were added ([i] offspring childhood maltreatment and [ii] maternal depression at 1- 4 years (early childhood)/11-16 years (late childhood). When the effects of offspring childhood maltreatment and maternal depression in early and late childhood were taken into account, the effect of prenatal maternal depression was reduced to a trend level of significance, Wald statistic = 3.0, p = .085, OR = 2.6, 95% CIs [.9, 5.7], model $\chi^2(4) = 20.3$, $p < .001$.

The same analytic procedure was performed, but using hierarchical multiple linear regression models, to assess whether prenatal maternal depression explained the variance in adult offspring depressive symptoms. At the first step, maternal marital status and offspring gender (both found to be associated significantly with depressive symptoms in preceding univariate analyses, see Table 9) were entered into the model. At the second step, prenatal maternal depression was added. A significant effect of prenatal maternal depression on adult offspring depressive symptoms was observed at this step, B = 1.6, t = 2.6, p = .011, 95% CI [.4, 2.8], model R^2 = .14, $F(3, 99) = 5.4$, p = .002. At the final step, offspring childhood maltreatment and exposure to maternal depression at 1-4 and/or 11-16 years were entered into the model. In the context of controlling for these childhood risk factors, the effect of prenatal maternal depression was reduced to non-significance, B = 1.0, t = 1.7, p = .10, 95% CI [-.2, 2.3], model R^2 = .20, $F(5, 96) = 4.8$, p = .001.

There are numerous possible interpretations to these findings. One plausible interpretation is that, as the univariate analyses revealed (i) that mothers who were depressed during pregnancy were highly likely to become depressed again during the offspring's childhood (see 3.1.2.3) and (ii) that a high degree of association exists between offspring childhood maltreatment and prenatal maternal depression (see Table 7), the fact that prenatal depression was found not to explain any significant variance in offspring adulthood depression (diagnoses or symptoms) when entered alongside offspring exposure to maternal depression post birth and childhood maltreatment may be indicative of confounding between prenatal maternal depression and the aforementioned childhood risk factors. Alternatively, prenatal depression may exert an effect that is indeed distinct to the effects of later childhood risks (maternal post-birth depression and offspring childhood maltreatment), yet the effects of these ensuing childhood insults may moderate and/or mediate the effects of prenatal maternal depression. Given the natural temporal sequence of gestation and childhood, this latter explanation warranted further investigation.

3.1.4. Is the effect of prenatal maternal depression on offspring depression influenced by environmental insults after birth?

To investigate the second hypothesis that psychosocial adversities during childhood contribute to the association between exposure to maternal depression during gestation and later life depressive psychopathology, the roles of (i) post-birth maternal depression and (ii) childhood maltreatment were assessed.

3.1.4.1. Independence of maternal post-birth depression and offspring childhood maltreatment

Maltreated offspring were no more likely than non-maltreated offspring to be exposed to maternal depression across their entire childhood (birth to 16), n = 102, $\chi^2(1) = 3.6$, p = .06, or specifically in early (1-4 years) and late childhood (11-16 years), n = 102, $\chi^2(1) = 1.9$, p = .17. However, partial correlation analyses revealed a significant positive association between offspring maltreatment and adulthood offspring depressive symptoms, n = 99, r_{pb} = .28, p = .022, when controlling for maternal depression in early and/or late childhood. Similarly, when controlling for offspring childhood maltreatment, there was a significant positive correlation between maternal depression in early and/or late childhood and offspring depressive symptoms, n = 99, r_{pb} = .25, p = .011. These findings suggest that maternal depression post-birth and childhood maltreated represent distinct insults that should be treated as separate risk variables when assessing the influence of environmental adversities occurring during childhood.

3.1.4.2. Moderation analysis: prenatal maternal depression on offspring adulthood depression by post-birth environmental insults

Moderation analyses are aimed at illustrating the conditions under which a purported effect may exist or be amplified or supressed. In order to test whether (i) maternal depression in early

and/or late childhood and (ii) offspring childhood maltreatment (M) moderated the effect of prenatal maternal depression (X) on offspring depression (Y), multiple regression analyses with interaction terms were added. The same analytic procedure was conducted for depressive diagnoses using hierarchical multiple logistic regression models, and for depressive symptoms using hierarchical multiple linear regression models. Two two-way interaction models were tested separately; the first model tested for an interaction of prenatal maternal depression by maternal depression in early and/or late childhood on offspring adulthood depression whilst controlling for offspring maltreatment (and associated sociodemographic characteristics), whilst the second model tested for an interaction of prenatal maternal depression by offspring childhood maltreatment on adult offspring depression, adjusted for maternal depression in childhood on offspring depression (and sociodemographic characteristics). In a final model, a three-way interaction of prenatal maternal depression by maternal depression in early/late childhood by offspring childhood maltreatment was tested. Results indicated no evidence of any statistically significant interactions for either adulthood offspring depression diagnoses or depressive symptoms.

3.1.4.3. Mediation analysis: prenatal maternal depression on offspring adulthood depression through maternal depression in childhood

The purpose of mediation analysis is to cast light on putative mechanisms by which an effect is exerted. In the case of longitudinal cohort studies, mediation analyses can be used to discern the pathways by which an effect is transmitted.

As mediation analytic techniques require the outcome variable to be continuous, all mediation analyses were conducted on offspring depressive symptoms, but not diagnoses. A mediation analysis using ordinary least squares path analysis was conducted to assess whether the effect of prenatal maternal depression (X) on adult offspring depressive symptoms (Y) was mediated by

the number of times offspring were exposed further to maternal depression after birth (1-4 years and/or 11-16 years) (M). These developmental time periods were selected on the basis that they were the only time periods in which exposure to maternal depression was significantly associated with offspring depressive symptoms (see Table 9). In all regression models, maternal marital status, offspring gender and offspring childhood maltreatment were entered as covariates (C_i). Inference about the statistical significance of the indirect effect was made through the construction of 95% bias-corrected bootstrap CI in which 10,000 bootstrap samples were generated. A visual illustration of this conceptual mediation model is presented in Figure 4 whilst the statistical results of the analyses are presented in Table 11.

The mediation analysis revealed that offspring exposure to maternal depression *in utero* indirectly led to increased offspring depressive symptoms in young adulthood through further offspring exposure to maternal depression in childhood. As can been seen in Table 11, prenatal maternal depression predicted cumulative exposure to maternal depression post birth (*a* = .45), and cumulative post-birth maternal depression predicted adult offspring depressive symptoms (*b* = .94). A 95% bias-corrected bootstrap CI for the indirect effect (*ab* = .42) based on 10,000 bootstrap samples was entirely above zero (.03, 1.15).

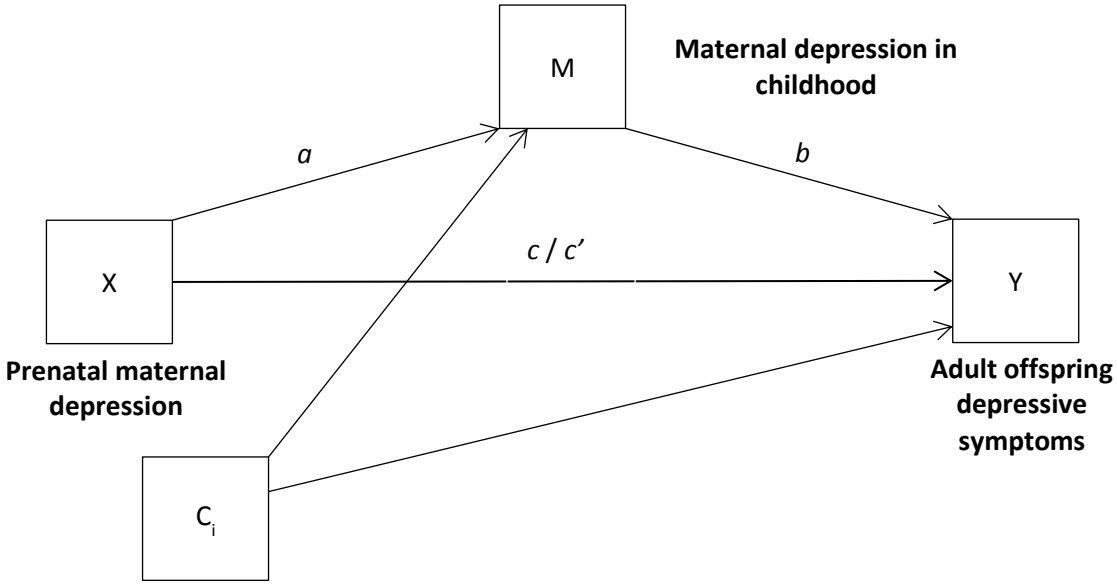

Figure 4. Conceptual mediation model for the effect of prenatal maternal depression (X) on adult offspring depressive symptoms (Y) through maternal depression in childhood (M), controlling for associated risks (C_i)

Table 11. Model coefficients for the mediation model of the effects of prenatal maternal depression on adult offspring depression mediated by maternal depression in childhood

Pathway	Coeff. (*SE*)	95% CI	Estimate (bootstrap *SE)*	95% bias corrected bootstrap CI
***c* (total effect)**	1.30 (.62)	**.07 – 2.53**		
a	.45 (.15)	**.16 – .75**		
b	.94 (.42)	**.09 – 1.78**		
***c'* (direct effect)**	.88 (.64)	-.39 – 2.14		
***ab* (indirect effect)**			.42 (.28)	**.03 – 1.15**

Note: *n* = 100. The regression coefficients presented are unstandardized B coefficients. All path estimates were calculated whilst controlling for maternal marital status (C_1), offspring gender (C_2) and offspring exposure to childhood maltreatment (C_3). An estimate of the total effect (*c* path coefficient) was obtained from the model that regressed Y on X, and excluded M (but controlled for all other associated risks). The indirect effect estimate (*ab)* is calculated as the product of the "*a*" and "*b*" path coefficients. Inference about the significance of the effect of this pathway (*ab*) was made through the calculation of 95% bias-corrected bootstrap CIs, whereby the number of bootstrap samples was set to 10,000. All regression models were significant at the $p < .01$ level.

3.1.4.4. Influences of childhood maltreatment

The next step of investigation was to determine whether offspring exposure to childhood maltreatment influenced the impact of prenatal maternal depression on adulthood offspring depression. Given that in prior analyses there was no evidence of an effect of either a two-way prenatal maternal depression by offspring childhood maltreatment, or three-way moderation (prenatal maternal depression by offspring childhood maltreatment by maternal depression post-birth) on offspring adulthood depression (see 3.1.4.2), I sought first to assess whether offspring childhood maltreatment (i) moderated the observed effect of prenatal maternal depression on offspring depressive symptoms through maternal depression in childhood (see Figure 5), or (ii) whether offspring maltreatment served as an independent mediator in the pathway between prenatal depression and offspring depressive symptoms (see Figure 6).

3.1.4.4.1 Conditional process analysis: moderated mediation

A diagram of the conceptual model for the moderated mediation model for the effect of prenatal maternal depression (X) on adult offspring depressive symptoms (Y) through maternal depression in early/late childhood (M), moderated by offspring childhood maltreatment (W) is depicted in Figure 5. A conditional process analysis using ordinary least squares path analysis was conducted, in which maternal marital status and offspring gender were entered as covariates (C_i) at all steps. In order to assess whether offspring childhood maltreatment moderated the observed effect of prenatal maternal depression through cumulative maternal depression in childhood, the *b* pathway was measured at each level of childhood maltreatment (absent versus present). This allowed for assessment of the conditional effect of M on Y at each level of W. Inference about the statistical significance of the conditional indirect effect was made through the construction of 95% bias-corrected bootstrap CIs in which 10,000 bootstrap samples were generated.

Results from the conditional process analysis did not reveal any significant interaction between cumulative maternal depression in childhood and offspring childhood maltreatment (simple effect: B = .91, *p* = .11; interactive effect: B = .40, *p* = .62). Indeed, 95% bias-corrected bootstrap confidence intervals for the conditional indirect effects (maltreatment absent versus maltreatment present) based on 10,000 bootstrap samples crossed zero [-.03, 1.37] and [-.01, 1.64], respectively. These data suggests that the mediated pathway from prenatal maternal depression to adult offspring depression through cumulative maternal depression post-birth is not moderated by the occurrence (or absence) of offspring childhood maltreatment.

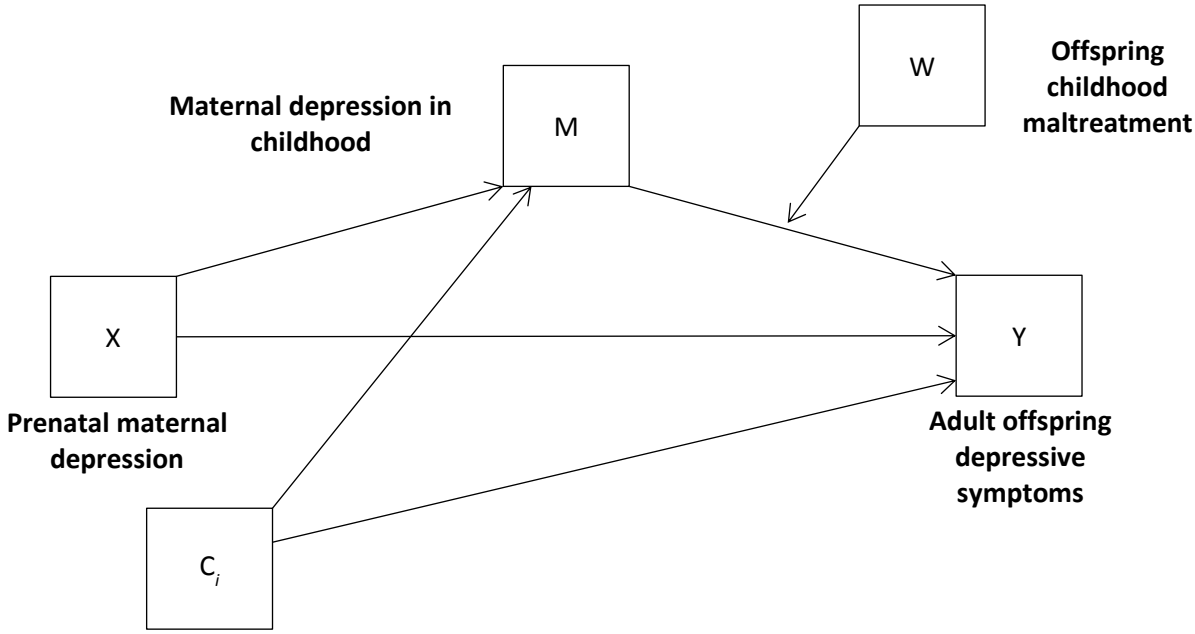

Figure 5. Conceptual moderated mediation model for the effect of prenatal maternal depression (X) on adult offspring depressive symptoms (Y) through maternal depression in childhood (M), moderated by offspring childhood maltreatment (W), and adjusted for associated risks (C_i)

3.1.4.4.2 Multiple mediation

I sought to investigate whether the effect of prenatal maternal depression on adult offspring depression was mediated by childhood maltreatment. A strong rational for this analysis comes from the results of prior univariate analyses: offspring childhood maltreatment was highly associated with both prenatal maternal depression and offspring depression (see 3.1.3.2 and 3.1.3.3). Given (i) that evidence of mediation of prenatal maternal depression through cumulative post-birth maternal depression was observed, and (ii) that maternal depression during early/late childhood is conceptually distinct from offspring childhood maltreatment (in addition to their effects being found to be independent, see 3.1.4.1), a multiple moderation model was tested in which offspring childhood maltreatment was conceptualised as a separate mediator to maternal post-birth depression, see Figure 6.

The multiple mediation analysis was conducted using ordinary least squares path analysis, with prenatal maternal depression entered as X, adult offspring depressive symptoms entered as Y and cumulative post-birth maternal depression entered as M_1. As mediation analysis also requires the mediator variables to be in continuous format, rather than dichotomous, a variable was constructed that summed the forms of maltreatment that each offspring had been exposed to. This cumulative maltreatment variable was entered into the analysis as M_2. In all regression models, maternal marital status and offspring gender were entered as covariates (C_i). Inference about the statistical significance of the indirect effects was made through the construction of 95% bias-corrected bootstrap CIs in which 10,000 bootstrap samples were generated.

The multiple mediation analysis revealed evidence of two indirect effects. That is, offspring exposure to maternal depression *in utero* indirectly led to increased offspring depressive symptoms in young adulthood through both further offspring exposure to maternal depression in early/late childhood and cumulative offspring childhood maltreatment. As can been seen in Table 12, prenatal maternal depression predicted both childhood risks (a_1 = .49, a_2 = .65). Both

cumulative post-birth maternal depression (b_2 = 1.01) and cumulative offspring childhood maltreatment (b_2 = .63) predicted adult offspring depressive symptoms. 95% bias-corrected bootstrap CIs for the two indirect effects (a_1b_1 = .50, a_2b_2= .42) were entirely above zero [.28, 1.90] and [.04, 1.09], respectively.

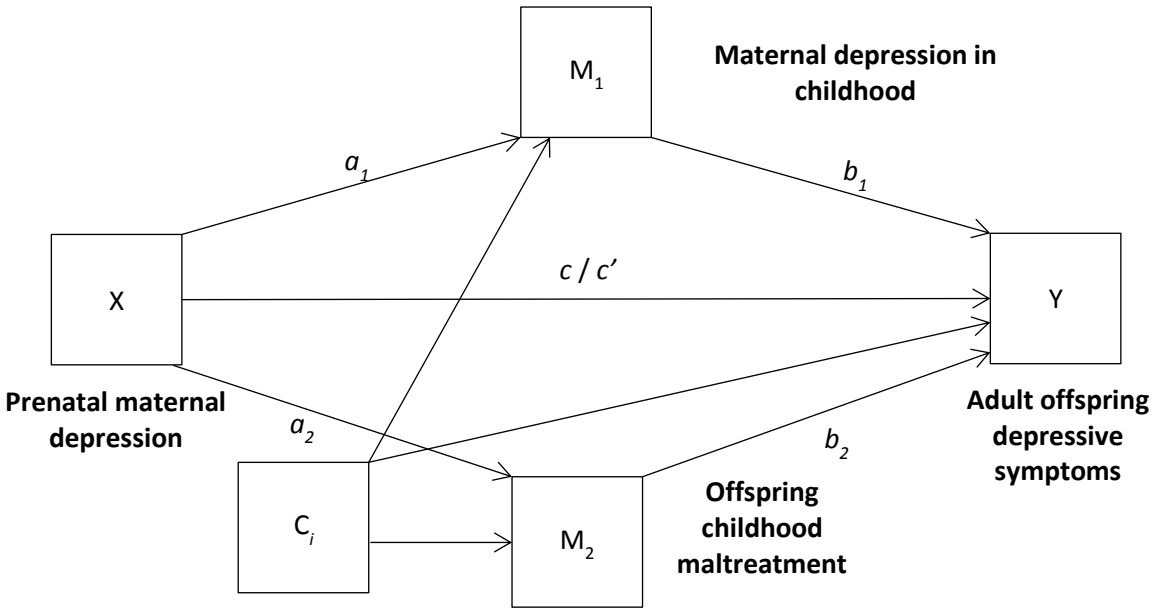

Figure 6. Conceptual multiple mediation model for the effect of prenatal maternal depression (X) on adult offspring depressive symptoms (Y) through maternal depression in childhood (M_1) and offspring childhood maltreatment (M_2), adjusted for associated risks (C_i)

Table 12. Model coefficients for the multiple mediation model of the effects of prenatal maternal depression on adult offspring depression mediated by (i) maternal depression in childhood (ii) offspring childhood maltreatment

Pathway	**Coeff. (*SE*)**	**95% CI**	**Estimate (bootstrap *SE*)**	**95% bias-corrected bootstrap CI**
***c* (total effect)**	1.53 (.61)	**.31 – 2.76**		
a_1	.49 (.14)	**.20 – .78**		
a_2	.65 (.21)	**.24 – 1.06**		
b_1	1.01 (.42)	**.19 – 1.84**		
b_2	.63 (.29)	**.06 – 1.21**		
***c'* (direct effect)**	.62 (.65)	-.67 – 1.91		
a_1b_1 (indirect effect of maternal post birth depression)			.50 (.30)	**.28 – 1.90**
a_2b_2 (indirect effect of offspring childhood maltreatment)			.42 (.25)	**.04 – 1.09**
Total indirect effect			.91 (.41)	**.28 – 1.90**

Note: *n* = 100. The regression coefficients presented are unstandardized B coefficients. All path estimates were calculated whilst controlling for offspring gender and maternal marital status. An estimate of the total effect (*c* path coefficient) was obtained from the model that regressed Y on X, and excluded M_1 and M_2 (but controlled for all other associated risks). Estimates of the indirect effects were calculated as the product of the respective "*a*" and "*b*" path coefficients for each pathway. Inference about the significance of the effects the direct paths were made through the calculation of 95% bias-corrected bootstrap CIs, whereby the number of bootstrap samples was set to 10,000. All regression models were significant at the $p < .01$ level.

3.1.4.5. Quantifying the impact of post birth environmental insults

To elaborate further on the finding that an offspring's exposure to psychosocial adversity after birth mediates the effects of prior exposure to maternal depression during gestation on her propensity to experience depression as a young adult, I examined the mean number of offspring depressive symptoms as a function of insult exposure. Given that exposure to maternal depression after birth and childhood maltreatment were observed to function as two distinct mediators, I assessed the mean number of depressive symptoms for these two pathways separately.

Turning first to the influence of maternal depression in childhood, offspring were categorised into the following four groups: (i) never exposed to maternal depression; (ii) exposure to only prenatal maternal depression; (iii) exposure to maternal depression in early/late childhood; (iv) exposure to maternal depression during gestation and during early and/or late childhood. The mean numbers of depressive symptoms in young adulthood were calculated for each group. Figure 7 displays these group means and standard errors (*SE*). As can be seen from Figure 7, offspring never exposed to maternal depression, $n = 40$, $M = 1.1$, $SD = 2.4$, had the lowest level of depressive symptoms. The mean number of depressive symptoms amongst offspring exposed only to maternal prenatal depression, $n = 10$, $M = 2.7$, $SD = 3.6$, or only maternal depression after birth, $n = 28$, $M = 2.5$, $SD = 3.2$ was similar. Offspring exposed to depression both before and after birth, $n = 35$, $M = 3.7$, $SD = 3.1$, had the highest level of adulthood depressive symptoms.

To test for group differences in symptom levels, a one-way ANOVA was performed, which yielded an overall significant effect, $F[3, 99] = 4.5$, $p = .005$. To account for differences in the sample size for each group, Gabriel's procedure was applied as *post hoc* analyses. These analyses revealed that offspring exposed to maternal depression in both pregnancy and childhood had significantly greater depressive symptoms (mean difference = 2.7, $SE = .7$, $p = .004$) in

comparison with offspring never exposed to maternal depression. Offspring exposed to maternal depression either only *in utero*, or only after birth, were not found to have significantly different levels of symptoms compared to (i) offspring who were never exposed, or (ii) compared to offspring exposed both before and after birth. This pattern of results complements those found in previous analyses (see 3.1.4.3). The finding that offspring exposed to both prenatal maternal depression and also maternal depression in childhood had significantly more adulthood depressive symptoms compared to offspring never exposed to maternal depression can be likened to the finding from the mediational analysis.

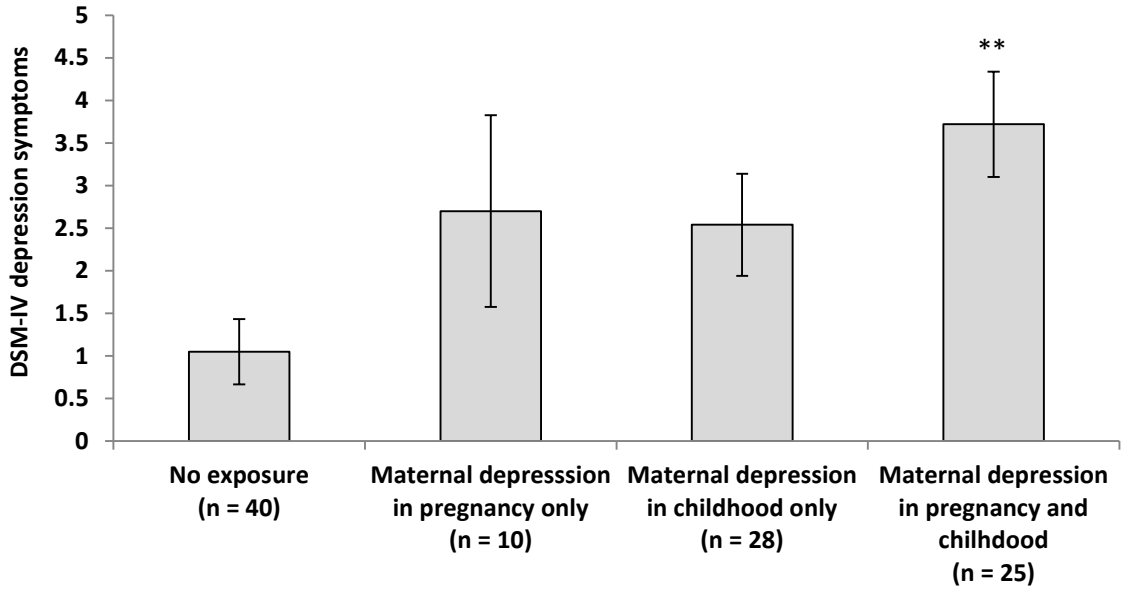

Figure 7. Mean adult offspring depressive symptoms as a function of timing of exposure to maternal depression, with *SE*

Note. **Offspring exposed to maternal depression in pregnancy and after birth have significantly higher levels of depressive symptoms in adulthood compared to offspring never exposed (p = .004).

Turning to the influence of childhood maltreatment, offspring were categorised into the following four groups: (i) not exposed to either prenatal maternal depression or childhood maltreatment; (ii) exposed to only prenatal maternal depression; (iii) exposed to only childhood maltreatment; (iv) exposed to prenatal maternal depression and childhood maltreatment. The mean numbers of depressive symptoms across young adulthood were calculated for each group, and are presented in Figure 8. As can be seen, offspring never exposed to any insult, n = 49, M = 1.2, SD = 2.5, had the lowest depressive symptoms in young adulthood. The mean number of adulthood depressive symptoms amongst offspring exposed to only maternal prenatal depression, n = 18, M = 3.1, SD = 3.1, or to only childhood maltreatment, n = 19, M = 3.0, SD = 3.4, was similar. Offspring exposed to both prenatal maternal depression and childhood maltreatment, n = 17, M = 3.8, SD = 3.4, had the highest mean adulthood depressive symptoms.

A one-way ANOVA revealed an effect of environmental insult, F[3, 99] = 4.7, p = .004. Gabriel's procedure revealed that offspring exposed to prenatal maternal depression and childhood maltreatment had significantly greater depressive symptoms in adulthood, mean difference = 2.6, SE = .8, p = .009, in comparison to offspring exposed to neither insult. There was no evidence for any statistical effect of cumulative exposure to both insults compared to one. Nor was there any evidence that offspring who were exposed to only one insult had any significant difference in the number of depressive symptoms they experienced in young adulthood in comparison to offspring who were exposed to neither insult.

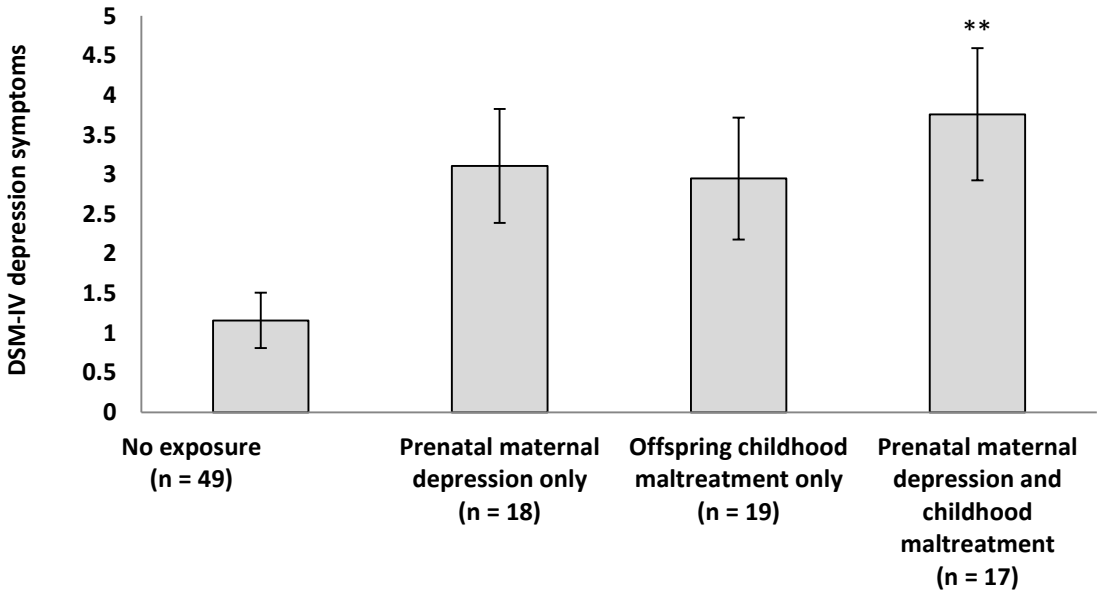

Figure 8. Mean adult offspring depressive symptoms as a function of exposure to early adversity, with *SE*

Note. **Offspring exposed to prenatal maternal depression and childhood maltreatment had a significantly higher number of depressive symptoms in adulthood compared to offspring never exposed to either insult (p = .009).

3.2. Part 2: Biological reactivity

3.2.1. Overview

The aim of the second part of the study was to investigate changes in biological systems in relation to exposure to prenatal maternal depression. Hypothalamus-pituitary-adrenal (HPA) axis activity, inflammation and metabolic status were measured at 25 years in order to evaluate reactivity in the respective neuroendocrinological, inflammatory and metabolic systems. The main goal was to evaluate whether offspring exposure to prenatal maternal depression was associated with reactivity in each biological system. A secondary goal was to assess the impact of childhood maltreatment on these systems' reactivity. First, descriptive analyses are provided for each biological system. Next, inter-associations between parameters of each system are examined. Univariate analyses are then performed to investigate the impact of concurrent stress and lifestyle and personal factors at 25 years on biological reactivity. I then assess the effects of prenatal maternal depression and childhood maltreatment on each system, followed by cumulative effect of adulthood depression. Multiple group comparisons are conducted in an attempt to distinguish the effects of early life stress from the effects of adulthood factors.

3.2.2. Descriptive analyses

3.2.2.1. HPA axis activity

The cortisol awakening response (CAR) was indexed through the measurement of salivary cortisol upon awakening, and at 15, 30 and 60 minutes post awakening. Cortisol levels at midday and at 8pm were collected in order to index the diurnal cortisol rhythm. Mean cortisol values at each time-point were calculated and plotted on two separate graphs: Figure 9 illustrates mean cortisol levels during the first hour after awakening, whilst Figure 10 illustrates mean cortisol levels across the day.

As can be seen from Figure 9, the mean cortisol level upon awakening was 8.9 nmol/L (*n* = 69, *SD* = 4.1). Cortisol levels rose during the first 15 minutes after awakening to a mean peak of 11.1 nmol/L (*n* = 69, *SD* = 4.4) at 15 minutes post awakening. They decreased slightly during the ensuing 15 minutes but remained relatively high with a mean level of 10.7 nmol/L at 30 minutes post awakening (*n* = 69, *SD* = 4.4). By 60 minutes post-awakening, the mean cortisol level had returned to a similar level as was observed upon awakening (*n* = 67, *M* = 8.2, *SD* = 4.8). To quantify the CAR further, area under the curve (AUC) calculations were performed for each participant, based on awakening, 15-, 30- and 60-minute cortisol levels. First, the total AUC based on the four respective time points was calculated, hereon referred to as the AUC with respect to ground (AUC_G), followed by the AUC with respect to awakening cortisol levels, hereon referred to as AUC with respect to increase (AUC_I). The mean AUC_G for cortisol levels across the first hour after awakening was 589.9 (*n* =67, *SD* = 211.1), whilst the mean AUC_I for cortisol levels in the hour following awakening was 60.1 (*n* =67, *SD* = 247.5). Collectively, these results provide an index for the CAR, which can be interpreted as reflecting the natural physiological responsiveness to the stress of awakening.

Turning to the diurnal cortisol rhythm, as can be seen from Figure 10, mean cortisol levels decreased through the morning to a mean level of 4.8 nmol/L (*n* = 69, *SD* = 3.7) at 12 pm, and further decreased through the rest of the day to a mean level of 2.1 nmol/L (*n* = 69, *SD* = 2.8) at 8pm. This pattern of results is consistent with the natural trend of decreasing cortisol levels through the course of the day. In order to quantify cortisol levels throughout the day, the AUC_G for awakening, 12pm and 8pm cortisol levels was calculated. The mean AUC_G for cortisol across the day was 3157.3 (*n* = 67, *SD* = 1555.7).

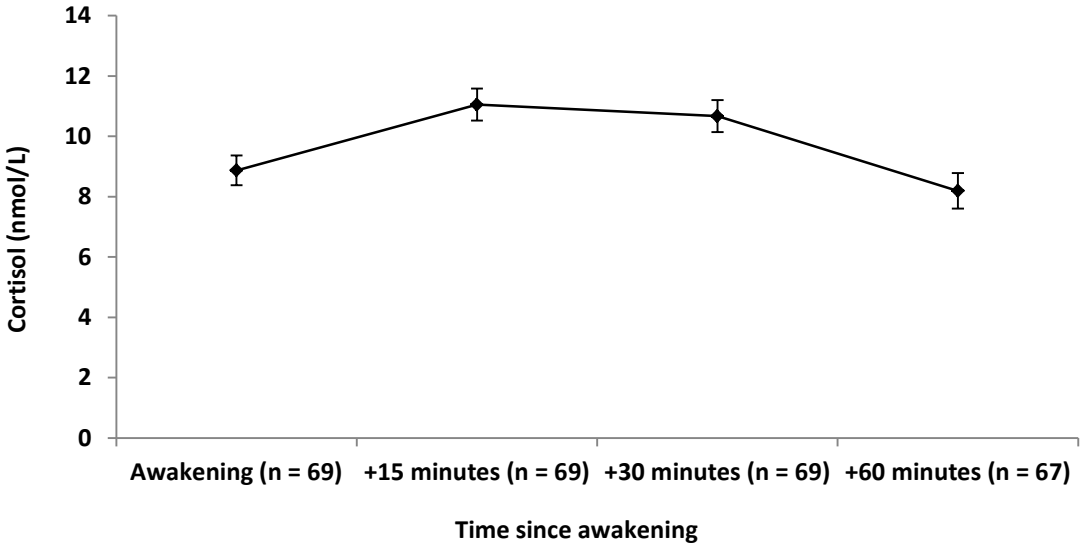

Figure 9. Cortisol awakening response as indexed by mean cortisol levels within the first hour after awakening, with *SE*

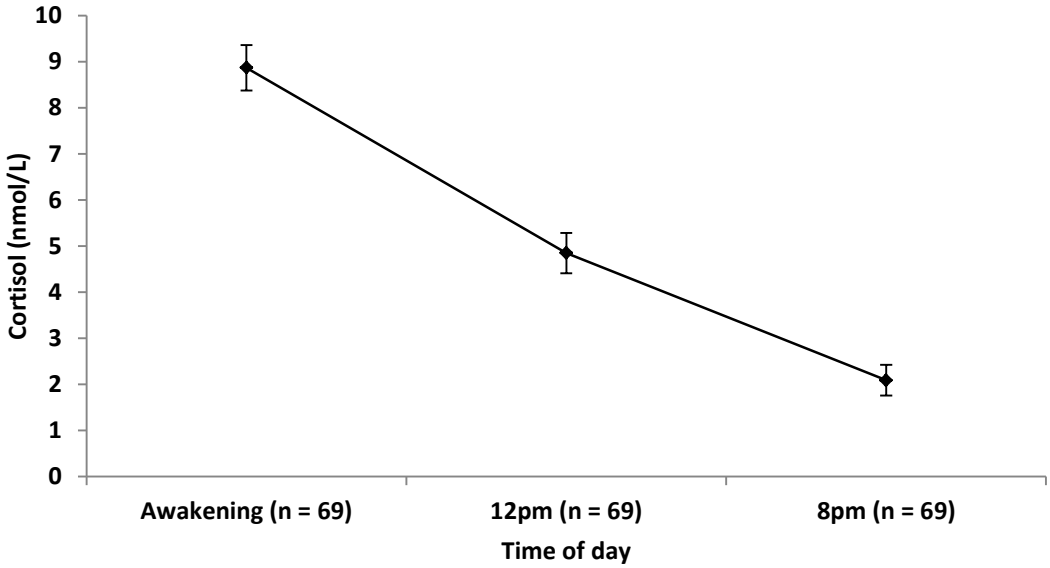

Figure 10. Diurnal cortisol levels as indexed by mean cortisol levels across the day, with *SE*

3.2.2.2. Inflammation

Plasma high sensitivity C-reactive protein (hsCRP) was measured as a biomarker of peripheral inflammation. The mean hsCRP level was 1.5 mg/L (*n* = 78, *SD* = 1.7). Thirteen (16.7%) offspring were categorised has having high levels of general inflammation (hsCRP ≥ 3.0), whilst 65 (83.3%) offspring were categorised as having normal levels of inflammation.

3.2.2.3. Metabolic parameters

Parameters associated with the metabolic syndrome were measured. Offspring's lipid profiles were assessed through the measurement of serum cholesterol (CHOL) and triglyceride (TG) levels. Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured as a means to quantifying levels of the respective lipoproteins. Plasma glycated haemoglobin (HbA1c) was measured to index regular plasma glucose levels, whilst body mass index (BMI) and waist circumference (WC) measurements provided an index of peripheral fat deposits. Mean metabolic parameter levels, along with frequencies for participants with clinically abnormal levels are presented in Table 13. The cumulative risk index marks the number of parameters that are abnormal.

Table 13. Descriptive statistics for metabolic parameters

Metabolic parameter	**Statistic**
CHOL (nmol/L)	*n* = 82
***M* (*SD*)**	4.5 (.8)
% > 5.0	20.7
TG (nmol/L)	*n* = 82
***M* (*SD*)**	1.2 (.8)
% > 2.0	9.8
HDL-C (nmol/L)	*n* = 82
***M* (*SD*)**	1.4 (.3)
% < 1.0	9.8
LDL-C (nmol/L)	*n* = 81
***M* (*SD*)**	2.5 (.8)
% > 3.0	23.5
HbA1c (%)	*n* = 81
***M* (*SD*)**	5.3 (.2)
% > 6.0	–
BMI (kg/m^2)	*n* = 102
***M* (*SD*)**	25.9 (6.2)
% > 30.0 (obese)	18.6
WC (cm)	*n* = 102
***M* (*SD*)**	88.4 (14.4)
Cumulative risks index	*n* = 81
***M* (*SD*)**	.8 (1.1)

Note. CHOL = cholesterol; TG = triglyceride; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; HbA1c = glycated haemoglobin; BMI = body mass index; WC = waist circumference.

3.2.2.4. Associations between cortisol, hsCRP and metabolic parameters

Exploratory intra-associations between parameters of each biological system were examined, along with the inter-associations across systems. Data for these univariate analyses are summarised in Table 14. Awakening cortisol levels were negatively correlated with delta statistics for cortisol levels at 30 and 60 minutes after awakening, respectively, as well as with AUC_I estimates for cortisol in the first hour. No associations were observed between awakening cortisol and cortisol levels at midday (12pm) and in the evening (8pm).

Inflammation levels were negatively correlated with the CAR, as indexed by significant negative correlations between hsCRP levels and delta statistics for cortisol at 30 minutes after awakening, and AUC_I estimates for cortisol in the first hour. In contrast, overall hsCRP levels were positively associated with BMI and WC.

Amongst metabolic parameters, BMI and WC were positively associated with CHOL, TG and LDL-C levels, and negatively correlated with HDL-C levels, suggesting an association between hyperlipidaemia and central obesity.

Table 14. Inter-correlations between offspring cortisol, hsCRP and metabolic parameters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	*n*
(1) Awakening cort.	–																69
(2) *Δ* +15 mins. cort.	-.17	–															69
(3) *Δ* +30 mins. cort.	-.41***	.65***	–														69
(4) *Δ* +60 mins. cort.	-.70***	.42***	.77***	–													67
(5) 12pm cort.	.04	.12	.05	.22†	–												69
(6) 8pm cort.	.13	-.16	-.33**	-.19	.17	–											69
(7) CAR AUC_G	.51***	.54***	.42***	.08	.11	-.10	–										67
(8) CAR AUC_I	-.50***	.69***	.95***	.87***	.16	-.26*	.39**	–									67
(9) Day cort AUC_G	.43***	-.04	-.19	-.13	.76***	.38**	.23†	-.12	–								67
(10) hsCRP	.14	-.04	-.33*	-.22†	.16	.11	-.11	-.29*	.18	–							78
(11) CHOL	.02	-.06	-.07	.02	-.09	-.15	-.07	-.06	-.11	.03	–						82
(12) TGs	.14	.07	.04	-.14	-.20	-.02	.18	-.02	-.07	.15	.46***	–					82
(13) HDL-C	-.14	-.02	.10	.23†	.28*	.23†	-.06	.14	.24†	-.22	-.09	-.40***	–				82
(14) LDL-C	.02	-.06	-.11	-.02	-.17	-.28*	-.07	-.09	-.21	.08	.87***	.24*	-.36**	–			81
(15) HbA1c	-06	-.10	-.13	-.10	-.16	.05	-.29*	-.15	-.14	.07†	.25*	.18	-.19†	.27*	–		81
(16) BMI	.23†	.07	-.27*	-.29*	-.02	.01	-.01	-.26*	-.03	.47***	.29**	.24*	-.50***	.40***	.01	–	102
(17) WC	.23†	.07	-.18	-.28*	-.19	-.10	-.04	-.23†	-.19	.39***	.32**	.44***	-.52***	.39***	.10	.82***	102

Note. Spearman's correlation coefficients are presented. Cortisol change statistics are the differences between the respective time points and awakening. Cort. = cortisol; mins. = minutes; CAR = cortisol awakening response; AUC_G = area under the curve with respect to ground; AUC_I = area under the curve with respect to increase; hsCRP = high sensitivity C-reactive protein; CHOL = cholesterol; TGs = triglycerides; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; HbA1c = glycated haemoglobin; BMI = body mass index; WC = waist circumference.

$^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

3.2.3. Univariate analyses between biological parameters and risk factors

As a first step to exploring reactivity in the respective biological systems as a function of early life adversity, univariate analyses were performed to assess potential associations between biological parameters and concurrent (at 25 years) lifestyle factors, stress and personal characteristics. Next, the impact of gross early life adversities, such as exposure to prenatal maternal depression and offspring childhood maltreatment were examined.

3.2.3.1. Associations with adulthood stress, lifestyle factors and personal characteristics

Indices of stress in adulthood comprised (i) current depression (DSM-IV depressive disorder diagnosis at 25 years), (ii) adulthood depression (DSM-IV depressive disorder diagnosis between 18 to 25 years), (iii) current depressive symptomatology (HAM-D) and (iv) recent stressful life events. Lifestyle factors included current (i) smoking, (ii) physical activity, (iii) alcohol consumption, (iv) fast food consumption and (v) medication use. Personal characteristics included (i) gender, (ii) ethnicity, (iii) educational attainment (iv) family of origin social class and (v) maternal marital status in pregnancy.

3.2.3.1.1 Adulthood stress

There was trend for a negative correlation between concurrent depression (DSM-IV disorders) and lower cortisol levels at 15 minutes post awakening, r_{pb} = -.23, p = .06. This association reached statistical significance for depression (DSM-IV diagnoses) during adulthood, 18-25 years; r_{pb} = -.24, p = .044. Delta cortisol at 15 minutes from awakening was negatively associated with adulthood depression, r_{pb} = -.30, p = .013. These findings suggest blunting of the CAR in offspring who experienced depression (DSM-IV diagnoses) recently. Current depression (DSM-IV diagnoses) was positively associated with CHOL levels, r_{pb} = .25, p = .026, and cumulative

metabolic risk index, r_{pb} = .24, p = .031. There were no associations between current or adulthood depression (DSM-IV diagnoses) and hsCRP levels. Depressive symptomatology (HAM-D) was not associated with any biological parameters. The number of current stressful life events was significantly negatively associated with overall hsCRP levels, r_s = -.25, p = .029.

3.2.3.1.2 Lifestyle factors

The use of medication with anti-inflammatory effects was negatively associated with cortisol levels at 15 and 30 minutes after awakening, r_{pb} = -.35, p = .003 and r_{pb} = -.24, p = .044, respectively, as well as with cortisol delta +15 minutes, r_{pb} = -.31, p = .010, and CAR AUC_G, r_{pb} = -.27, p = .025. Notably, there was no evidence of statistical outliers in the above mentioned raw cortisol values in participants who used medication. This would suggest that medication use did not significantly affect cortisol levels. There was a trend for a positive association between medication use and overall hsCRP levels, r_{pb} = .22, p = .06. Medication use was not associated with any metabolic parameters. Exercise frequency was negatively correlated with BMI, WC and cumulative metabolic risks index, r_s = -.29, p = .003, r_s = -.32, p = .001 and, r_s = -.26, p = .021, respectively. Frequency of alcohol consumption was also negatively correlated with BMI, r_s = -.27, p = .008. Fast food consumption was positively correlated with HbA1c levels, r_s = .23, p = .045. Smoking was not associated with any biological parameters.

3.2.3.1.3 Personal characteristics

Offspring education was positively associated with HDL-C levels and cumulative metabolic risks index, r_{pb} = .27, p = .015 and r_{pb} = .23, p = .038, respectively, and negatively associated with LDL-C levels, r_{pb} = -.23, p = .037. There was a significant association between ethnicity and both TG and WC, whereby not being white British was positively associated with larger WCs, r_{pb} =.23, p = .020, and higher TG levels, r_{pb} = .29, p = .008. Gender was also significantly associated with TG levels,

WC and HDL-C levels, whereby girls had lower TGs, r_{pb} = -.35, p = .001, smaller WCs (r_{pb} = -.21, p = .039) and higher HDL-C levels, r_{pb} = .28, p = .010. Family of origin social class was positively associated with the cumulative metabolic risks index, r_{pb} = .29, p = .009. Maternal unmarried status in pregnancy was positively correlated with TG levels, r_{pb} = .26, p = .020 and AUC_G diurnal cortisol levels, r_{pb} = .32, p = .009.

3.2.3.2. Associations with distal risk factors

The primary purpose of the next set of analyses was to test the hypothesis that prenatal maternal depression and offspring childhood maltreatment modulate biological changes in HPA axis function, peripheral inflammation levels and parameters associated with metabolic syndrome. In addition to performing univariate analyses to test the effects of (i) prenatal maternal depression and (ii) childhood maltreatment, I also tested for effects of (iii) maternal prenatal drinking, (iv) maternal prenatal smoking, (v) maternal prenatal anxiety, (vi) medical problems during pregnancy, (vii) gestational age, (viii) birth weight, (ix) breastfeeding and (x) exposure to maternal depression from birth to 16.

Minimal group differences were observed amongst the main predictors. No group differences were observed between offspring exposed to prenatal maternal depression compared to those not so exposed. There were no differences in the proportion of maltreated offspring with clinically high (≥ 3.0mg/L) inflammation compared to non-maltreated offspring, but maltreated offspring, *M*, = 1.2, *SD* = 1.2, were found to have lower overall levels of hsCRP compared to non-exposed offspring, *M*, = 1.6, *SD* = 1.8, z = -2.0, p = .042. Statistics for these analyses are presented in Table 15.

Additional distal risk factors had no effects on hsCRP levels. Amongst cortisol levels, exposure to prenatal maternal smoking was positively correlated with AUC_G diurnal cortisol levels, r_s = .26, p = .037, whilst exposure to prenatal drinking was positively correlated with evening cortisol levels, r_{pb} = .28, p = .020. Exposure to prenatal maternal anxiety was positively correlated with cortisol levels at 12pm. r_s = .25, p = .041, and negatively associated with HbA1c levels, r_{pb} = -.29, p = .008. Medical problems during pregnancy were positively correlated with LDL-C levels, r_{pb} = .23, p = .040. Lengthier breastfeeding was positively correlated with HDL-C levels, r_s = .29, p = .039, and negatively correlated with BMI and WC, r_s = -.31, p = .018 and r_s = -.28, p = .034, respectively.

Table 15. Univariate analyses between prenatal maternal depression, offspring childhood maltreatment and biological parameters at 25 years

	Exposure to prenatal maternal depression *M* (*SD*)			**Exposure to childhood maltreatment *M* (*SD*)**		
	None *n* = 44-67[a]	**Exposed *n* = 21-35**[a]	**Group effect**	**None *n* = 33-67**[a]	**Exposed *n* = 26-35**[a]	**Group effect**
Cortisol (nmol/L)						
Awakening, *M*	8.8 (3.8)	9.0 (4.7)	*p* = .98	8.8 (3.9)	9.0 (4.5)	*p* = .70
+15 mins, *M*	11.1 (4.4)	11.0 (4.5)	*p* = .86	11.0 (4.3)	11.1 (4.7)	*p* = .64
+30 mins, *M*	10.6 (4.6)	10.7 (4.2)	*p* = .62	10.2 (3.9)	11.4 (5.2)	*p* = .25
+60 mins, *M*	8.4 (5.4)	7.7 (3.3)	*p* = .79	7.9 (5.2)	8.7 (4.2)	*p* = .27
Δ* +15 mins, *M	2.3 (3.4)	2.0 (4.4)	*p* = .72	2.2 (6.7)	2.1 (3.8)	*p* = .98
Δ* +30 mins, *M	1.8 (5.1)	1.8 (5.5)	*p* = .82	1.4 (5.2)	2.4 (5.4)	*p* = .57
Δ* +60 mins, *M	-.3 (6.8)	-1.2 (6.4)	*p* = .89	-.9 (7.1)	-.3 (6.1)	*p* = .59
CAR AUC_G, *M*	590.4 (220.9)	588.9 (194.6)	*p* = .80	570.2 (2.2)	621.0 (243.3)	*p* = .52
CAR AUC_I, *M*	64.3 (239.6)	51.5 (268.7)	*p* = .68	46.2 (248.0)	82.0 (250.2)	*p* = .52
12pm, *M*	5.2 (4.2)	4.1 (1.9)	*p* = .57	5.2 (3.8)	4.4 (3.3)	*p* = .37
8pm, *M*	1.9 (1.5)	2.6 (4.6)	*p* = .55	2.5 (3.5)	1.5 (.8)	*p* = .52
Day AUC_G	3200.0 (1620.0)	3063.6 (1438.4)	*p* = .86	3310.3 (1755.7)	2915.9 (1165.7)	*p* = .50
Inflammation						
hsCRP, *M* (mg/L)	1.3 (1.7)	1.9 (1.8)	*p* = .46	1.6 (1.8)	1.2 (1.2)	***p* = .042**
% ≥ 3.0	12.0	25.0	*p* = .21[b]	18.4	13.8	*p* = .76[b]
Metabolic						
CHOL, *M* (nmol/L)	4.5 (.8)	4.4 (.8)	*p* = .24	4.5 (.8)	4.5 (.9)	*p* = .92
TGs, *M* (nmol/L)	1.1 (.6)	1.4 (1.1)	*p* = .58	1.3 (.9)	1.1 (.6)	*p* = .89
HDL-C, *M* (nmol/L)	1.5 (.4)	1.3 (.3)	*p* = .25	1.5 (.4)	1.4 (.3)	*p* = .28
LDL-C, *M* (nmol/L)	2.6 (.8)	2.4 (.7)	*p* = .38	2.4 (.6)	2.7 (.9)	*p* = .25
HbA1c, *M* (%)	5.3 (.3)	5.3 (.2)	*p* = .37	5.3 (.2)	5.3 (.2)	*p* = .38
BMI, *M* (kg/m^2)	26.1 (6.9)	25.4 (4.7)	*p* = .94	25.9 (6.4)	25.8 (5.8)	*p* = .96
WC, *M* (cm)	88.4 (15.3)	88.4 (12.6)	*p* = .88	88.8 (15.0)	87.6 (13.4)	*p* = .84
Cumulative risks, *M*	.7 (1.0)	.9 (1.3)	*p* = .98	.7 (1.0)	.9 (1.3)	*p* = .43

Note. Group effects were based upon the independent samples *t*-test for associations that permitted parametric analysis, and the Mann-Whitney test for associations that did not permit parametric analysis. Cortisol change statistics are the differences between the respective time points and awakening. CAR = cortisol awakening response; AUC_G = area under the curve with respect to ground; AUC_I = area under the curve with respect to increase; hsCRP = high sensitivity C-reactive protein; CHOL = cholesterol; TGs = triglycerides; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; HbA1c = glycosylated haemoglobin; BMI = body mass index; WC = waist circumference.

[a] *n*s vary due to missing data. [b] Fisher's exact test applied due to one contingency table cell showing an expected cell count less than five.

3.2.4. Group comparisons for distal risks stratified by adulthood depression

One potential explanation for a lack of group differences on biological parameters between offspring exposed to prenatal maternal depression versus those not so exposed, and between offspring exposed to maltreatment versus those who did not experience maltreatment, could be due to modulation by later insults in adulthood that are known to be correlated with changes in the biological systems (i.e. depression).

To test this premise, a split group analysis was performed in order to conduct between group comparisons of distal risks separately for depressed and non-depressed adult offspring. A variable indicating whether offspring had ever experienced a depressive disorder during adulthood (18-25 years), including current depressive symptomatology at 25, years was used to split the sample. Between-group comparisons for prenatal maternal depression and offspring childhood maltreatment were conducted separately for depressed and non-depressed offspring. Results from these analyses are presented in Table 16 and Table 17. In instances where significant effects were observed within a single group, moderation analyses and ANOVAs were then performed on the whole sample to qualify these findings further.

Table 16. Group comparisons of exposure to prenatal maternal depression and childhood maltreatment amongst offspring not depressed in adulthood

	Non-depressed adult offspring					
	Exposure to prenatal maternal depression *M* (*SD*)			Exposure to childhood maltreatment *M* (*SD*)		
	None *n* = 35-48[a]	Exposed *n* = 9-14[a]	Group effect	None *n* = 33-46[a]	Exposed *n* = 11-16[a]	Group effect
Cortisol (nmol/L)						
Awakening, *M*	8.8 (3.3)	9.3 (4.7)	*p* = .84	8.7 (3.4)	9.4 (4.0)	*p* = .45
+15 mins, *M*	11.6 (4.2)	13.0 (3.9)	*p* = .23	11.3 (4.4)	13.6 (3.0)	*p* = .07
+30 mins, *M*	10.8 (4.5)	12.4 (3.8)	*p* = .16	10.1 (3.8)	14.3 (4.6)	***p* = .004**
+60 mins, *M*	8.3 (5.3)	8.6 (4.7)	*p* = .60	7.7 (5.4)	10.2 (3.8)	***p* = .018**
Δ* +15 mins, *M	2.8 (3.0)	3.8 (5.0)	*p* = .56	2.6 (3.4)	4.2 (3.4)	*p* = .19
Δ* +30 mins, *M	2.0 (5.0)	3.1 (7.2)	*p* = .54	1.4 (5.1)	4.9 (5.8)	*p* = .07
Δ* +60 mins, *M	-.4 (6.9)	-.6 (8.1)	*p* = .94	-.9 (7.4)	.8 (6.0)	*p* = .50
CAR AUC_G, *M*	596.8 (203.2)	672.6 (176.7)	*p* = .21	566.4 (185.0)	750.0 (179.5)	***p* = .004**
CAR AUC_I, *M*	74.2 (227.3)	117.2 (346.8)	*p* = .54	48.8 (185.0)	185.5 (252.3)	*p* = .10
12pm, *M*	5.0 (4.0)	4.0 (1.4)	*p* = .87	5.3 (4.1)	3.3 (1.0)	*p* = .15
8pm, *M*	1.8 (1.4)	4.1 (6.9)	*p* = .70	2.5 (3.7)	1.3 (1.1)	*p* = .18
Day AUC_G, *M*	3136.9 (1645.9)	3400.6 (1937.6)	*p* = .37	3398.3 (1865.5)	2544.5 (667.3)	*p* = .21
Inflammation						
hsCRP, *M* (mg/L)	1.3 (1.2)	2.8 (2.8)	*p* = .08	1.6 (1.6)	1.8 (2.5)	*p* = .10
% ≥ 3.0	10.8	41.7	***p* = . 029**[b]	16.2	25.0	*p* = . 67[b]
Metabolic						
CHOL, *M* (nmol/L)	4.4 (.7)	4.2 (.7)	*p* = .37	4.4 (.7)	4.4 (.6)	*p* = .65
TGs, *M* (nmol/L)	1.2 (.6)	1.2 (.6)	*p* = .58	1.2 (.6)	1.2 (.6)	*p* = .37
HDL-C, *M* (nmol/L)	1.4 (.4)	1.4 (.2)	*p* = .81	1.4 (.4)	1.3 (.3)	*p* = .25
LDL-C, *M* (nmol/L)	2.5 (.7)	2.3 (.7)	*p* = .71	2.4 (.7)	2.5 (.7)	*p* = .59
HbA1c, *M* (%)	5.3 (.2)	5.3 (.2)	*p* = .78	5.3 (.2)	5.4 (.2)	*p* = .08
BMI, *M* (kg/m^2)	27.0 (7.5)	26.6 (5.5)	*p* = .89	26.9 (7.1)	27.0 (7.3)	*p* = .98
WC, *M* (cm)	90.7 (16.7)	89.8 (12.5)	*p* = .86	89.9 (16.5)	92.3 (13.8)	*p* = .28
Cumulative risks, *M*	.7 (1.0)	.5 (.9)	*p* = .59	.7 (1.0)	.7 (1.1)	*p* = .97

Note. Group effects were based upon the independent samples *t*-test for associations that permitted parametric analysis, the Mann-Whitney test for associations that did not permit parametric analysis, and Pearson's χ^2 test for independence for associations with two dichotomous variables. Cortisol change statistics are the differences between the respective time points and awakening. CAR = cortisol awakening response; AUC_G = area under the curve with respect to ground; AUC_I = area under the curve with respect to increase; hsCRP = high sensitivity C-reactive protein; CHOL = cholesterol; TG = triglyceride; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; HbA1c = glycosylated haemoglobin; WC = waist circumference.

[a] *n*s vary due to missing data. [b] Fisher's exact test applied due to one contingency table cell showing an expected cell count less than five.

Table 17. Group comparisons of exposure to prenatal maternal depression and childhood maltreatment amongst offspring who experienced depression in adulthood

	Depressed adult offspring					
	Exposure to prenatal maternal depression *M* (*SD*)			Exposure to childhood maltreatment *M* (*SD*)		
	None *n* = 10-19[a]	Exposed *n* = 12-21[a]	Group effect	None *n* = 7-21[a]	Exposed *n* = 15-19[a]	Group effect
Cortisol (nmol/L)						
Awakening, *M*	9.0 (5.6)	8.7 (5.0)	*p* = .89	9.2 (5.7)	8.7 (5.0)	*p* = .95
+15 mins, *M*	9.4 (4.8)	9.5 (4.4)	*p* = .94	9.8 (3.7)	9.3 (5.0)	*p* = .95
+30 mins, *M*	10.0 (5.0)	9.6 (4.2)	*p* = .78	10.7 (4.4)	9.3 (4.6)	*p* = .41
+60 mins, *M*	8.9 (6.2)	7.1 (1.9)	*p* = .94	8.6 (4.5)	7.5 (4.3)	*p* = .35
Δ* +15 mins, *M	.4 (4.1)	.8 (3.5)	*p* = .94	.6 (4.6)	.6 (3.3)	*p* = .90
Δ* +30 mins, *M	1.0 (5.7)	.8 (4.1)	*p* = .94	1.5 (5.8)	.6 (4.3)	*p* = .34
Δ* +60 mins, *M	-.1 (7.1)	-1.6 (5.3)	*p* = .44	-.5 (6.2)	-1.1 (6.2)	*p* = .71
CAR AUC_G, *M*	567.9 (285.9)	531.0 (191.2)	*p* = .83	585.8 (214.9)	526.4 (244.9)	*p* = .49
CAR AUC_I, *M*	29.7 (289.4)	6.1 (201.9)	*p* = .89	35.6 (271.9)	6.1 (227.4)	*p* = .35
12pm, *M*	5.8 (4.8)	4.1 (2.2)	*p* = .35	4.4 (2.6)	5.1 (4.1)	*p* = .91
8pm, *M*	2.2 (1.5)	1.5 (.7)	*p* = .33	2.2 (1.9)	1.6 (.6)	*p* = .74
Day AUC_G, *M*	3427.1 (1585.3)	2810.9 (932.2)	*p* = .60	2882.7 (1073.5)	3188.2 (1385.9)	*p* = .87
Inflammation						
hsCRP, *M* (mg/L)	1.2 (1.4)	1.2 (1.6)	*p* = .79	1.8 (2.0)	.8 (.8)	*p* = .10
% ≥ 3.0	15.4	12.5	*p* = .98[b]	25.0	5.9	*p* = .28[b]
Metabolic						
CHOL, *M* (nmol/L)	5.0 (1.0)	4.5 (.9)	*p* = .07	4.7 (.8)	4.7 (1.1)	*p* = .63
TGs, *M* (nmol/L)	1.1 (.4)	1.5 (1.4)	*p* = .84	1.7 (1.5)	1.1 (.6)	*p* = .37
HDL-C, *M* (nmol/L)	1.5 (.3)	1.3 (.4)	*p* = .14	1.5 (.4)	1.4 (.3)	*p* = .57
LDL-C, *M* (nmol/L)	3.0 (.9)	2.5 (.8)	*p* = .13	2.5 (.5)	2.8 (1.0)	*p* = .49
HbA1c, *M* (%)	5.4 (.3)	5.3 (.2)	*p* = .15	5.3 (.3)	5.3 (.2)	*p* = .71
BMI, *M* (kg/m^2)	23.7 (3.9)	24.8 (4.1)	*p* = .50	23.8 (4.0)	24.8 (4.0)	*p* = .57
WC, *M* (cm)	82.7 (9.2)	87.5 (12.9)	*p* = .51	86.5 (11.1)	83.8 (11.9)	*p* = .30
Cumulative risks, *M*	.8 (.8)	1.1 (1.6)	*p* = .86	.7 (1.0)	1.1 (1.4)	*p* = .58

Note. Group effects were based upon the *t*-test for associations that permitted parametric analysis, the Mann-Whitney test for associations that did not permit parametric analysis, and Pearson's χ^2 test for associations with dichotomous variables. Cortisol change statistics are the differences between the respective time points and awakening. CAR = cortisol awakening response; AUC_G = area under the curve with respect to ground; AUC_I = area under the curve with respect to increase; hsCRP = high sensitivity C-reactive protein; CHOL = cholesterol; TG = triglyceride; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; HbA1c = glycosylated haemoglobin; WC = waist circumference.

[a] *n*s vary due to missing data. [b] Fisher's exact test applied due to one contingency table cell showing an expected cell count less than five.

3.2.4.1. Cortisol

3.2.4.1.1 Prenatal maternal depression

Findings from the split group analysis did not reveal any statistical effects of prenatal maternal depression on offspring cortisol levels in either the non-depressed or depressed group. Descriptively however, amongst non-depressed offspring, those exposed to prenatal maternal depression showed a propensity for a higher CAR compared to non-depressed offspring, as indexed by elevated mean cortisol levels at 15 and 30 minutes post awakening. Within the depressed group, this inclination was not observed; rather, mean cortisol levels at 15 and 30 minutes after awakening were lower amongst depressed offspring, irrespective of exposure to prenatal maternal depression. Tests of moderation across the whole sample did not reveal any interaction between prenatal maternal depression and offspring adulthood depression on any cortisol measurements. Figure 11 presents mean cortisol levels for the CAR as a function of depression during adulthood and exposure to prenatal maternal depression.

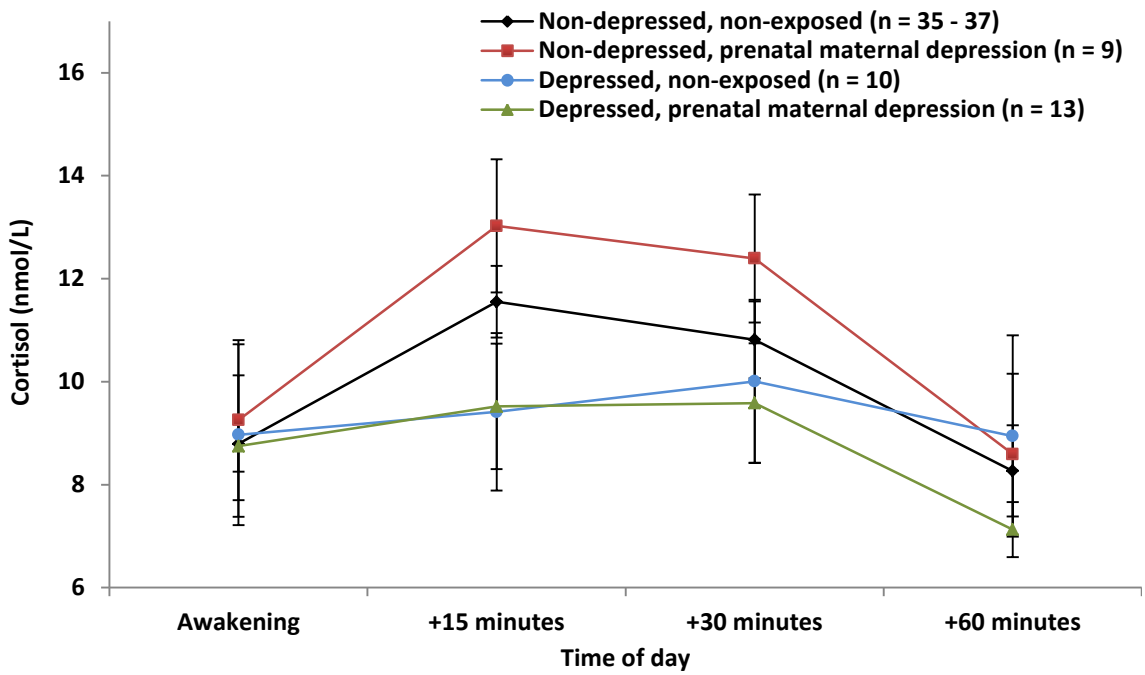

Figure 11. Cortisol awakening response as a function of exposure to prenatal maternal depression and depression in adulthood, with *SE*

3.2.4.1.2 Childhood maltreatment

The CAR, as indexed by mean cortisol levels for maltreated and non-maltreated offspring split by depression status in adulthood, is depicted in Figure 12. Findings from the split group analysis revealed that amongst offspring who were not depressed in adulthood, those who were maltreated exhibited a higher levels of cortisol at 30 minutes, n = 11, M, = 14.3, SD = 4.6, z = -2.9, p = .004, and 60 minutes, n = 11, M, = 10.2, SD = 3.8, z = -2.4, p = .018, post awakening, and a significantly larger CAR AUC_G, n = 11, M, = 750.0, SD = 179.5, z = -2.9, p = .004, compared to offspring who were not maltreated, n = 35, M, = 10.1, SD = 3.9, n = 33, M, = 7.7, SD = 5.4 and n = 33, M, = 566.4, SD = 185.0, respectively. There was also a trend for maltreated offspring to have higher cortisol levels at 15 minutes, n = 11, M, = 13.6, SD = 3.0, z = -1.8, p = .07, and a larger delta at 30 minutes post-awakening, n = 11, M, = 4.2, SD = 3.4, z = -1.8, p = .07, compared to non-maltreated offspring, n = 35, M, = 11.3, SD = 4.4 and n = 35, M, = 2.6, SD = 3.4, respectively.

In contrast, there were no statistical differences in the CAR between maltreated and non-maltreated offspring who had become depressed during adulthood. Descriptively, depressed offspring exhibited a flatter CAR, irrespective of maltreatment status. Moderation analyses conducted across the whole sample revealed a significant interaction between childhood maltreatment and adulthood depression on cortisol levels at 30 minutes post awakening, B = -5.2, t = -2.2, p = .028, 95% CI [-9.8, -.58], model R^2 = .18, F(4, 64) = 3.6, p = .010, controlling for covariates (anti-inflammatory medication, see 3.2.3.1.2), and on CAR AUC_G, B = -243.0, t = -2.1, p = .036, 95% CI [-469.2, -16.8], model R^2 = .12, F(3, 63) = 2.9, p = .04. These analyses also confirmed the conditional effect of exposure to maltreatment on 30-minute cortisol levels, B = 4.0, t = 2.8, p = .007, 95% CI [1.1, 6.8], and CAR AUC_G, B = 183.6, t = 2.6, p = .011, 95% CI [42.76, 324.37], amongst only those offspring who were not depressed in adulthood. Interaction terms for cortisol levels and 60 minutes post awakening did not meet statistical significance, B = -3.6, t = -1.4, p = .18, 95% CI [-9.0, 1.7].

To evaluate further the modulatory effects of adulthood depression, one-way ANOVAs were performed (in the whole sample) to compare (i) non-depressed non-maltreated, (i) non-depressed maltreated, (ii) depressed non-maltreated, and (iv) depressed maltreated offspring. There was a significant main effect for cortisol levels and 30 minutes, $F[3, 65] = 3.4$, $p = .021$, and for CAR AUC_G, $F[3, 63] = 2.9$, $p = .040$. Gabriel's procedure *post hoc* analyses revealed that offspring exposed to childhood maltreatment but who were not depressed in adulthood had significantly higher 30-minute cortisol (mean difference = 4.2, $SE = 1.5$, $p = .024$) and CAR AUC_G (mean difference = 183.6, $SE = 70.4$, $p = .011$) in comparison to those non-depressed and non-maltreated, and in comparison to those maltreated but also depressed (mean difference = 5.0, $SE = 1.7$, $p = .021$ and mean difference = 223.4, $SE = 80.3$, $p = .04$, respectively). These data complement the findings from the split analyses suggesting hyperactivity in the cortisol response system amongst maltreated (but not depressed) offspring. Furthermore, they also add to these findings by revealing that the experience of depression in conjunction with childhood maltreatment leads to blunting of the CAR. In terms of diurnal cortisol levels, no statistical differences were observed amongst measures of cortisol through the day, in either the split group analysis or moderation analyses.

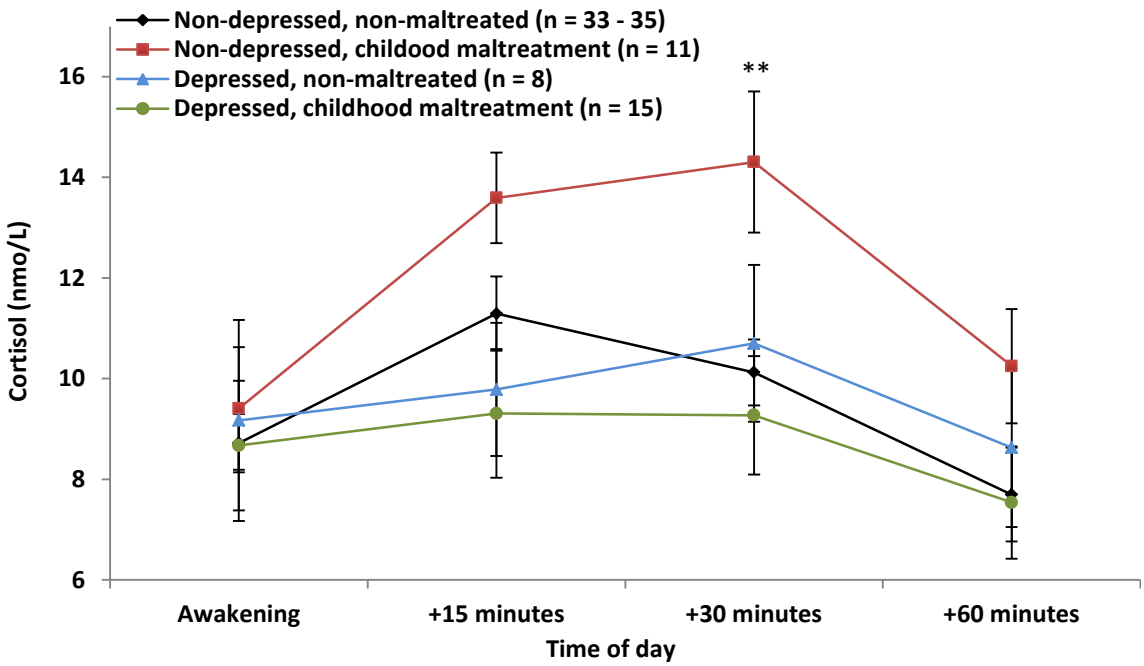

Figure 12. Cortisol awakening response as a function of exposure to childhood maltreatment and depression in adulthood, with *SE*

Note. **Cortisol levels at 30 minutes post awakening were significantly higher amongst non-depressed, maltreated offspring in comparison with non-depressed, non-maltreated offspring (p = .024), and in comparison with depressed, maltreated offspring (p = .021).

3.2.4.2. Inflammation

3.2.4.2.1 Prenatal maternal depression

Results from the split group analysis revealed that amongst offspring who were not depressed in adulthood, there was a trend for those exposed to prenatal maternal depression to have higher overall hsCRP levels, *n* = 12, *M*, = 2.8, *SD* = 2.8, than those not so exposed, *n* = 37, *M*, = 1.3, *SD* = 1.2, *z* = -1.7, *p* = .08. Notably, a significantly greater proportion (41.7%) of offspring exposed to prenatal maternal depression had clinically high (≥ 3.0 mg/L) hsCRP compared to offspring not so exposed, 10.1%, $\chi^2(1) = 5.8$, *p* = .029, *OR* = 5.9, 95% CI [1.3, 27.7]. These statistics are presented in Figure 13. In contrast, amongst offspring who were depressed in adulthood, there were no statistical group differences in hsCRP levels between offspring exposed and not exposed to prenatal maternal depression. A moderation analysis on the whole sample revealed a trend for an interaction between prenatal maternal depression and offspring depression in adulthood on overall hsCRP levels, B = -1.4, *t* = -1.7, *p* = .09, 95% CI [-2.98, .01], model $R^2 = .16$, $F(4, 73) = 3.4$, *p* = .01, controlling for covariates (recent stressful life events, see 3.2.3.1.1).

3.2.4.2.2 Childhood maltreatment

There was no effect of exposure to childhood maltreatment on hsCRP levels within either non-depressed or depressed adult offspring. Moderation analyses did not reveal any evidence of a statistically significant interaction.

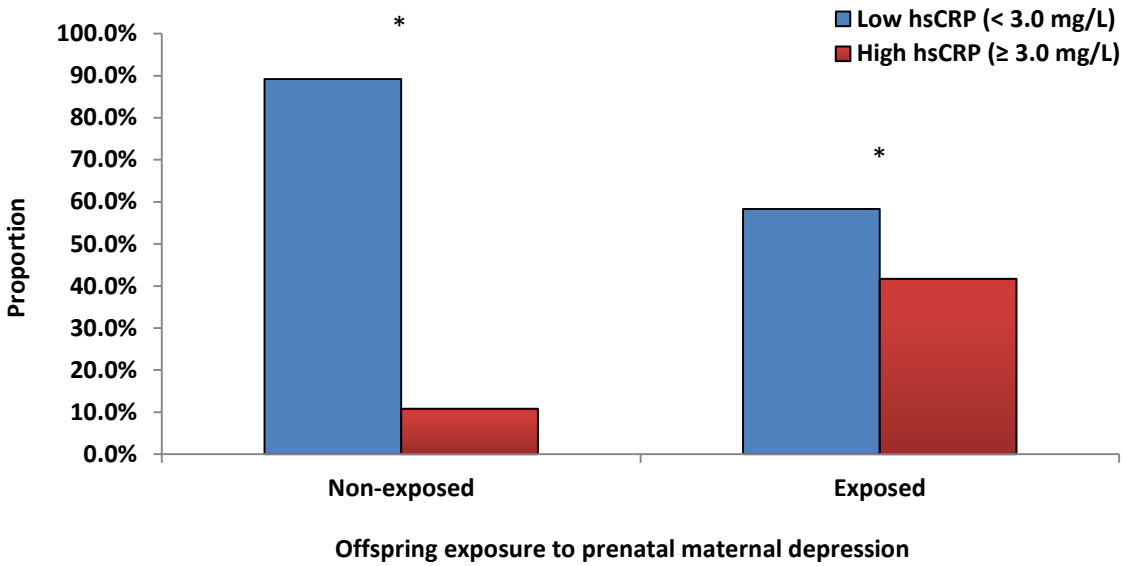

Figure 13. Proportions of non-depressed adulthood offspring with clinically high and low hsCRP as a function of exposure to prenatal maternal depression.

Note. *A significantly greater proportion of offspring exposed to prenatal maternal depression had clinically high (≥ 3.0 mg/L) hsCRP compared to offspring not so exposed (p = .029).

3.2.4.3. Metabolic parameters

3.2.4.3.1 Prenatal maternal depression

Findings from the split analysis did not reveal any effects of prenatal maternal depression on any metabolic parameters in either group. Similarly, there was no evidence of any interaction between prenatal maternal depression and offspring adulthood depression across the whole sample.

3.2.4.3.2 Childhood maltreatment

The split group analysis revealed a trend for a positive significant effect of childhood maltreatment on HbA1c levels amongst offspring not depressed in adulthood, z = -1.7, p = .08. A moderation analysis on the whole sample revealed a trend for an interaction between offspring childhood maltreatment and offspring depression in adulthood on HbA1c levels, B = -.2, t = -1.8, p = .08, 95% CIs [-.43, .02], model R^2 = .16, F(5, 71) = 2.6, p = .032, controlling for covariates (prenatal anxiety in pregnancy and offspring concurrent fast food consumption, see 3.2.3).

CHAPTER 4: DISCUSSION

4.1. Overview

This thesis used a prospective longitudinal design to investigate the psychological and biological adulthood consequences of offspring exposure to maternal depression during gestation. First, results of the present study, the 25-year offspring follow-up, as a continuation of the prospective longitudinal South London Child Development Study (SLCDS), are summarised. Subsequent sections embed these findings into a broader literature context, highlight strengths and limitations of the study, as well as provide suggestions for future research. Finally, clinical implications are discussed.

4.2. Summary of main findings

4.2.1. Adulthood depression

The findings from this study demonstrate for the first time using a prospective design, that offspring exposure to maternal depression *in utero* predicts clinical depression (DSM-IV MDD, dysthymic disorder and depression NOS) during young adulthood (18-25 years). This association is independent of the effects of other associated maternal stressors during pregnancy as well as offspring characteristics. Moreover, the data reveals this to be a robust effect that exists for both categorical diagnoses of depressive disorders and symptom levels. These findings provide clear support for the 1st hypothesis of an association between prenatal maternal depression and offspring adulthood depression.

When considering the impact of further environmental adversities occurring after birth, the results demonstrate that both offspring exposure to childhood maltreatment and maternal depression during the childhood years, influence the effect of prenatal maternal depression on offspring adulthood depression. That is, when taking these post-birth psychosocial adversities into account, prenatal maternal depression alone does not independently predict offspring

adulthood depression; rather, offspring childhood maltreatment and post-birth maternal depression are found individually to mediate this. Two sets of analyses provide support for this finding: (i) mediation analyses and (ii) ANOVA analyses.

These results reveal that mothers who were depressed in pregnancy are more likely to (i) expose their child to greater levels of childhood maltreatment, and (ii) have further depressive episodes during the offspring's childhood. Both of these psychosocial adversities subsequently compound the risk of the offspring becoming depressed in adulthood. In particular, post-birth environmental adversities are found to be distinct psychosocial mechanisms for the transmission of gestational stress exposure into risk for depressive psychopathology in young adulthood. Overall, these data provide partial support for the second and third hypotheses. Whilst the effect of prenatal maternal depression on offspring adulthood depression is not found to be fully independent of post-birth environmental insults (hypothesis 2), childhood maltreatment is found to contribute detrimentally to this effect (hypothesis 3).

4.2.2. Biological sequelae

At 25 years, a blunted cortisol awakening response (CAR) was correlated with higher overall inflammation (high sensitivity C-reactive protein; hsCRP) levels. Amongst metabolic parameters, visceral fat deposition (body mass index [BMI], waist circumference [WC]) was positively correlated with serum lipid levels (cholesterol [CHOL], triglycerides [TGs], low-density lipoprotein cholesterol [LDL-C]). High-density lipoprotein cholesterol (HDL-C) levels were negatively correlated with TG levels and physical obesity indices. There was also a positive association between higher plasma glucose levels (indexed by glycosylated haemoglobin; HbA1c) and serum lipids (CHOL, LDL-C). These results demonstrate significant cohesion amongst metabolic syndrome risk parameters. Furthermore, higher visceral fat deposits were positively correlated with inflammation levels, but negatively correlated with cortisol levels. These findings

provide support for hypothesis 4: alterations in cortisol levels, inflammation and metabolic parameters are associated.

4.2.2.1. Hypothalamic-pituitary-adrenal axis

With regard to the ramifications of exposure to maternal depression *in utero* on cortisol levels at 25 years, no significant effects were observed. Thus, no support was found for hypothesis 5, that prenatal maternal depression predicts altered cortisol levels at 25 years. However, offspring who experienced depression during adulthood had lower cortisol levels as indexed by a blunted CAR. These data provide partial support for the 6th hypothesis, as they indicate a link between adulthood depression and altered cortisol levels. However, this finding was not influenced by a history of exposure to prenatal maternal depression. The 7th hypothesis proposed that exposure to childhood maltreatment would be associated with altered cortisol levels. Results revealed that non-depressed offspring with a history of childhood maltreatment exhibited elevated HPA axis activity, as indexed by an increased CAR, whilst depressed maltreated offspring exhibited a blunting of the CAR. These findings suggest two important points: (i) that maltreatment leads to dysregulation in cortisol activity, showing hyperactivity in the absence of adulthood depression, and (ii) that depressive psychopathology contributes cumulatively to dysregulation in the HPA axis as observed through blunting of the CAR.

4.2.2.2. Inflammation

Results demonstrated that a significantly greater proportion of non-depressed adult offspring who were exposed to prenatal maternal depression had clinically high (≥ 3.0 mg/L) inflammation at 25 years in comparison to non-depressed non-prenatally exposed offspring. When testing for this association amongst depressed offspring, no evidence for an effect was found. These findings provide partial support for the 8th hypothesis – prenatal maternal depression predicts

adult offspring inflammation – but do not provide support for the 9th hypothesis: depression in adulthood will contribute cumulatively to this effect. The 10th hypothesis proposed that a history of childhood maltreatment would predict elevated inflammation at 25 years. Results revealed no significant differences in the proportion of offspring with clinically high inflammation as a function of maltreatment exposure. However, in regards to overall hsCRP levels, offspring not exposed to childhood maltreatment were found to have higher overall hsCRP levels compared to non-maltreated offspring.

4.2.2.3. Metabolic parameters

Findings show that offspring experiencing depression (DSM-IV diagnosis) currently at 25 years had elevated total CHOL levels, as well as a greater number of cumulative metabolic risk parameters, elevated to a clinically abnormal range (indexed by collective metabolic risks index). This provides partial support for the 12th hypothesis: combined early life stress and adulthood depression will result in the greatest abnormalities in metabolic function. However, there was no evidence that exposure to depression during gestation or to childhood maltreatment affected metabolic function at 25 years; thus, hypotheses 11 – prenatal maternal depression predicts 25-year metabolic abnormalities – and 13 – childhood maltreatment predicts metabolic abnormalities at 25 years – were not supported.

4.3. Prenatal maternal depression, offspring childhood maltreatment and offspring depression in young adulthood

The finding that offspring exposure to depression *in utero* predicts depression in adulthood is a completely novel finding that has not previously been found in any prospective investigations. It extends previous findings from our South London Child Development study which have shown that prenatal maternal depression predicts offspring depressive psychopathology (Pawlby et al., 2009) as well as externalising psychopathology (Hay et al., 2010) in childhood. These results are also consistent with the wealth of clinical studies that have demonstrated an association between prenatal maternal stress (mood disorders and stressful experiences during pregnancy) and offspring emotional psychopathology in childhood (Glover, 2011; O'Donnell, 2010; Van Den Bergh et al., 2005; Van den Bergh et al., 2008). Notably, the prevalence of depressive disorders amongst adult offspring was fairly high. This finding is consistent with prevalence rates of common mental disorders in an urban sample. Indeed, Hatch and colleagues (2012) recently found a four-fold greater proportion of depressive disorders amongst residents of south east London in comparison with residents across the rest of the England. It is also possible that this high prevalence could reflect intergenerational transmission of depression given the high prevalence of maternal depression in this cohort.

Furthermore, these results extend our previous finding in the South London Child Development Study, that mothers who are depressed during pregnancy are more like to have offspring who go on to experience childhood physical, sexual abuse and harsh discipline (although not necessarily perpetrated by the mother) at 11 years (Pawlby et al., 2011). They demonstrate that prenatal maternal depression predicts not only physical and sexual abuse, but also emotional abuse, as well as cumulative abuse and neglect up to 17 years of age. These findings are also in line with recent findings using data from the Avon Longitudinal Study of Parents and Children (ALSPAC) which have revealed that maternal depression and anxiety during pregnancy predict victimisation in middle childhood (Lereya & Wolke, 2013). These results inform the discussion

about child-driven and maternal-driven mechanisms that likely potentiate this association. The fact that offspring who were exposed to maternal depression during gestation reported significantly greater emotional abuse than non-prenatally exposed offspring suggests that mother-driven effects are just as likely to play a part as play as offspring-elicited punitive parenting due to a more externalising temperament: an initial explanation for this association. The fact that fathers accounted for a large proportion of the perpetrators of parental emotional abuse and neglect does not stand in opposition with a mother-driven effects model. It is quite plausible that in instances of paternal emotional abuse, mothers are struggling to protect their offspring from this emotional abuse by their partner. Furthermore, our data are more in line with clinical and animal findings which show that prenatally stressed mothers express lower maternal care and attachment-related behaviours and attitudes towards their offspring (Alhusen, 2008; Champagne & Meaney, 2006; Feldman et al., 2007; Stronach et al., 2011). Whether this finding generalises to other forms of prenatal maternal stress remains to be seen; yet, this finding identifies prenatal maternal depression as a clear and robust risk factor for child maltreatment potential.

The finding that the effects of prenatal maternal depression on offspring depressive psychopathology in adulthood were mediated by offspring exposure to childhood maltreatment are also consistent with our previous finding that sexual and physical abuse modulated the association between prenatal maternal depression and offspring externalising and internalising psychopathology at 16 years (Pawlby et al., 2011). It is interesting to note that contrary to our findings regarding offspring psychopathology at 16 years, at 25 years we did not find an effect of prenatal maternal depression that was independent of the effects of offspring exposure to childhood maltreatment. There are several possible reasons for this. One reason could be that the definition of child maltreatment in this present study was much broader, encompassing emotional abuse and neglect as well as sexual and physical abuse, and also spanned a greater time period (birth to 17 years). These differences suggest that the present findings might reflect

a more comprehensive account of the role of maltreatment, which was not captured in our earlier work when examining the role of sexual and physical abuse and harsh discipline.

These differences could also reflect changes in the nature of psychopathology between childhood and adolescence. Indeed, depression tends to rise through the transition from adolescence to young adulthood (Costello et al., 2011) as well as being preceded by externalising problems in earlier childhood (Hipwell et al., 2011; Plant et al., 2013). This would indicate that these findings at 25 years are likely indexing a different form of psychopathology to our findings at 16 years. It is also worth noting that at 16 years we assessed depression and conduct disorder, whilst at 25 years we assessed only depression. The reason for this is that in childhood antisocial behaviour psychopathology such as conduct disorder is classified as an axis I disorder, whilst in adulthood antisocial behaviour is classified as an axis II personality disorder, such as antisocial personality disorder. Interestingly, the present study's findings are also in line with those from the only other prospective study that has investigated the modulatory effects of environmental adversity on the link between prenatal maternal depression and offspring emotionality. Sharp and colleagues (H Sharp et al., 2012) found that prenatal maternal depression predicted infant negative emotionality only in offspring whose mothers showed low maternal care (low stroking behaviour)

The finding that the effect of prenatal maternal depression is mediated by offspring childhood maltreatment is also consistent with the wealth of clinical and animal studies demonstrating an association between childhood adversity and depressive psychopathology in young adulthood (Antonia Bifulco et al., 2002; Green et al., 2010; Keyes et al., 2012; Nanni et al., 2012; Widom et al., 2007). The present data add to these findings not only by replicating this robust association, but by extending them through highlighting the remarkable similarity in outcomes between offspring exposed to prenatal maternal depression and offspring exposed to childhood maltreatment, as well as finding evidence for a maltreatment-mediated effect. Moreover, they

support the notion that prenatal maternal depression and childhood maltreatment are part of the same putative pathway linking early life environmental adversity (gestational and childhood) to risk for depression in adulthood.

The results of the present study also demonstrate the important role of maternal depression after birth, through the offspring's childhood years, on offspring depression in adulthood. The data demonstrate that depression in the child's early (4-11 years) and late (11-16 years) childhood predicted depression during adulthood. These findings are consistent with prospective epidemiological and clinical studies that demonstrate an effect of maternal post-birth depression on offspring depressive psychopathology (E Barker, Copeland, Maughan, Jaffee, & Uher, 2012; Halligan, Murray, Martins, & Cooper, 2007; Mars et al., 2012; Murray et al., 2011). Notably however, we find that the impact of post-birth maternal depression plays a mediating role in the pathway between prenatal maternal depression and offspring adulthood depression. None of the aforementioned studies included measurement of maternal psychopathology during pregnancy. Given the strong comorbidity between maternal depression during pregnancy and after birth it is likely that many of the reported effects of postnatal depression were also indexing the potential confounding effects of prenatal maternal depression that went unmeasured. It is also interesting to note that the effects of maternal depression after birth have been shown to index further environmental risk factors such as low socioeconomic status, low education and family adversity, and that when these factors are taken into account, the effect size of maternal depression per se is dramatically reduced (E Barker et al., 2012). This suggests that the mediated effect of prenatal maternal depression on offspring adulthood depression by maternal post-birth depression characterises risk from mothers who experience recurrent depression themselves as well as wider familial adversity.

Overall, the observed links between prenatal maternal depression, offspring childhood maltreatment and offspring adulthood psychopathology cast light on the natural course of the

development of depressive disorders as a consequence of maternal depression during pregnancy. The fact that the data are from a naturalistic birth cohort study is testament to the immense ecological validity of these findings. They illuminate two putative pathways linking offspring exposure to prenatal maternal depression with depressive disorders in young adulthood: (i) the cumulative and mediating effects of exposure to childhood maltreatment and (ii) the aggregating effect of further exposure to maternal depression after birth. The fact that there is a mediated effect of exposure to prenatal maternal depression on offspring adulthood depression, with no direct effect of prenatal maternal depression that is independent of the influence of psychosocial adversities after birth means that these data do not conclusively support a foetal programming hypothesis. Rather, these data cast light on the developmental trajectories linking prenatal maternal depression to offspring adulthood depression. It is likely, given a sample size of 103 and the high correlation between exposure to insults prenatally (i.e. maternal prenatal depression) and postnatally (i.e. maternal depression in childhood and adolescence, offspring childhood maltreatment) that the absence of an independent direct effect is an artefact of a low number of cases with only prenatal maternal depression and adulthood depression and no further insults after birth.

4.4. Reactivity in biological systems

4.4.1. Correlations between HPA axis activity, inflammation and metabolic parameters

The finding that a blunted CAR response is correlated with elevated overall inflammation levels is consistent with the literature that proposes that dysregulation in the HPA axis system and inflammatory systems are interlinked (A Miller, 2009). Indeed, theory postulates that under normal circumstances the HPA axis system and inflammatory system exist in balance with one another and chronic stress can disrupt this balance in favour of inflammatory processes at the expense of glucocorticoid receptor (GR) signalling (Horowitz et al., 2013). As a blunted CAR is indicative of impaired GR-mediated negative feedback, a process commonly referred to as GC resistance, this data supports this theoretical premise by demonstrating that abnormalities in GC response to the stress of awakening are correlated with elevated hsCRP levels, a marker of systemic inflammation. According to this theoretical account, these data are indicative of stress-induced dysfunction in both systems. Findings for the strong inter-association between amplified physiological parameters related to metabolic function are in line with the notion of the metabolic syndrome as a clustering of risk factors for cardiovascular disease (CVD). My data show that elevated CHOL, TGs, LDL-C, HbA1c, BMI and reduced HDL-C were associated. These have been identified previously as markers of the metabolic syndrome and risk factors for CVD (Beck-Nielsen, 1999; Ford, 2005; International Diabetes Federation, 2006; World Health Organization, 1999). With regards to the relationship between metabolic parameters and inflammation and HPA axis function, central obesity (high BMI, large WC) was positively correlated with overall hsCRP levels and negatively correlated with cortisol levels. This former finding is consistent with existing theory that proinflammatory cytokines are overexpressed by adipose tissues in cases of obesity (Emanuela et al., 2012; Hotamisligil et al., 1993). Obesity thereby represents a state of chronic low-grade inflammation (Leonard, 2013).

GCs have been found to lead to hyperglycaemia, obesity dyslipidaemia and hypertension (Reynolds, 2013). Abnormalities in HPA axis function are thought to contribute to cardio-metabolic risks through hormonal and psychosocial pathways. It has been argued that stress-induced HPA axis changes can precipitate increase in the intake of high-caloric food (Dallman et al., 2003), which may represent one the psychosocial mechanisms linking HPA axis dysfunction to adult obesity. In terms of biological mechanisms, Dinan and colleagues (2004) reviewed a series of studies demonstrating that excessive HPA axis activity is associated with elevated visceral fat deposition, whereby this link was argued to be mediated by the antagonising effects on insulin action, thereby inducing insulin resistance (Reynolds & Walker, 2003) and the promoting effects on lipoprotein lipase, leading to an increase of storage of TGs and CHOL in adipocytes. It is conceivable that all of these mechanisms are at play amongst offspring in this study, which could account for the correlation between a blunted cortisol response and visceral obesity. It is also possible that interplay between inflammation, HPA axis dysregulation and obesity resulted in aggregated enhancing effects on one another.

4.4.2. Biological consequences of depression in adulthood

The present findings demonstrate that offspring who were depressed during young adulthood (18-25 years) show a blunted cortisol response to awakening. A dysregulated HPA axis is a well documented component of MDD (Pariante & Lightman, 2008). Functional impairment of the glucocorticoid receptor (GR) is theorised to be the key candidate mechanism for this dysregulation which results in overall functional impairment in HPA axis activity that has most commonly been observed to manifest as hypercortisolemia (Bhagwagar et al., 2003, 2005; Nemeroff & Vale, 2005; Vreeburg et al., 2009). Yet, hypoactivity has also been observed (Jarcho et al., 2013). Whilst these findings might seem paradoxical, impaired negative feedback (as evidenced through reduced suppression of cortisol following dexamethasone administration) was observed amongst patients who exhibited a hyperactive CAR as well as amongst those who

exhibited a blunted CAR. This would suggest that (i) HPA axis dysregulation is the core neurobiological facet of depression, (ii) that cortisol levels are merely one index of this wider dysregulation and (iii) that the manifestation of this dysregulation in terms of abnormalities in cortisol secretion is likely to be modulated by numerous other related factors such as illness severity and chronicity as well as other personal characteristic (e.g. age, previous psychiatric history), all of which varied in these studies. In light of these facts, the blunted CAR observed in this study is viewed as an index of overall HPA axis dysfunction that most likely reflects a state of GC resistance. However, without measures of other parameters of GC resistance, such as data from a cortisol suppression test, or more direct assessments of GR function like the DNA expression status of the GR, the precise underlying mechanisms of impairment remain speculative.

The finding that currently (at 25 years) depressed offspring exhibited a greater number of clinically elevated metabolic parameters is consistent with existing literature that documents high comorbidity between depression and obesity, CVD and the metabolic syndrome (Barth et al., 2004; Luppino et al., 2010; Pan et al., 2012). Given the cross-sectional nature of this finding, with both metabolic parameters and current depression measured concurrently at 25 years, it is not possible to draw conclusions on the directionality of these effects. Nevertheless, this finding shows that young adults with a current clinically diagnosed DSM-IV depressive disorder exhibit comorbid components of the metabolic syndrome, which are also risk factors for CVD. Interestingly, there was no evidence of an association between elevated metabolic parameters and the experience of depression earlier during young adulthood (i.e. 18-25 years). One could speculate, therefore, that the ramifications of depression on the metabolic profile are short-lived, whereby changes that may have occurred with the experience of earlier depression have dissipated after remission. However, drawing conclusions based on negative findings is unjustified, so this remains an anecdotal observation rather than scientific conclusion.

4.4.3. Biological sequelae of early life stress

The impact of early life adversity, namely exposure to prenatal maternal and offspring childhood maltreatment, on biological reactivity was modulated by the experience of depression in young adulthood. Findings revealed that a significantly greater proportion of offspring who had been exposed to prenatal maternal depression had clinically high levels of systemic inflammation at 25 years compared to offspring who were not so exposed, yet this association was only observed amongst offspring who did not experience depression during adulthood. One interpretation is that for offspring who did not experience depression during adulthood, these data are indicative of the influences of these distal factors in the absence of the impact of more proximal adulthood stress (i.e. depression during young adulthood). In contrast, the biological systems of offspring who experienced depression in young adulthood would be reflective of the impact of depression, given that alterations in the three respective biological systems are reported amongst depressed individuals (see 1.9.2, 1.9.6, 1.9.10). Indeed in the present data, offspring depression per se was associated with abnormalities in HPA axis function and metabolic function. Following this logic, the present findings could be interpreted as demonstrating the novel association between prospectively assessed exposure to depression during gestation and elevated inflammation 25 years later in adult life. This notion is consistent with recent findings from animal and human studies demonstrating that prenatal maternal depression is associated with a dysregulated immune response and systemic inflammation (Diz-Chaves et al., 2013; Entringer, Kumsta, et al., 2008; O'Connor, Winter, et al., 2013). It is hypothesised that prenatally depressed mothers would have elevated inflammation and HPA axis activity, both of which could operate as candidate mechanisms for the programming of foetal immune reactivity and HPA axis-inflammatory dysregulation.

In line with this premise, we would predict that offspring exposed to prenatal maternal depression would also exhibit dysregulation in HPA axis activity. In the current findings, there was no statistical effect for the influence of exposure to prenatal maternal depression on HPA

axis activity, although there was a pattern for non-depressed offspring exposed to prenatal maternal depression to show an elevated CAR.

In contrast, there was a significant effect of offspring exposure to childhood maltreatment on HPA axis function, as indexed by a heightened CAR amongst non-depressed maltreated offspring and a blunted CAR amongst depressed maltreated offspring. This data is in line with existing human and animal literature documenting abnormalities in HPA axis activity as a function of exposure to maltreatment as well as the experience of depression (Bhagwagar et al., 2005; De Bellis et al., 1994; de Kloet & Oitzl, 2003; Francis et al., 1999; Heim et al., 2002; Jarcho et al., 2013; Pariante & Lightman, 2008). One possible explanation for these findings is that they reflect the impact of cumulative exposure to chronic psychosocial adversities. That is, in the absence of adulthood depression, maltreatment is associated with hypercortisolemia, which likely reflects a state of GC resistance. In individuals who experienced both childhood maltreatment and depression during adulthood, the hypoactivity in the HPA axis may reflect severe dysregulation and system exhaustion. Indeed, several studies have demonstrated that in the context of chronic trauma hypoactivity is observed (Hart et al., 1996; G. Miller, Chen, & Zhou, 2007).

This account is in line with the theoretical premise of biological sensitivity to context (BSC) advocated by Ellis and colleagues (2008). BSC theory proposes that *adaptive phenotypic plasticity*, the capacity for a single genotype to support a range of phenotypes in response to particular ecological conditions, is malleable to the environmental conditions within which an organism develops. Importantly, BSC articulates a U-shaped neurobiologically-mediated sensitivity to context which enables an individual to match their biological and behavioural systems to the parameters of their developmental environments. Ellis and colleagues (2005) first found evidence for this curvilinear phenomenon when studying stress reactivity in children exposed to varying degrees of stressful family environments. They observed that the level of stress reactivity in the children followed a U-shaped pattern as the level of enrichment in the

environment decreased and adversity increased. BSC could therefore explain why maltreated children who differ in their experience of later depression show stress responses of opposite valence.

An alternative explanation for the opposing effects of maltreatment amongst depressed and non-depressed offspring could be that the blunting observed amongst maltreated depressed offspring could reflect the overall dysregulation (in this case hypoactivity) that was observed amongst all depressed offspring. That is, the influence of this proximal stress, depression, could "trump" alterations precipitated by early insults. Nevertheless, irrespective of this latter interpretation whereby the biological sequelae in depressed maltreated individuals reflects perpetuated stress exposure or the effects of adulthood depression per se, the fact that non-depressed maltreated offspring exhibited a significantly different CAR from non-depressed non-maltreated offspring, suggests that exposure to maltreatment itself results in neurobiological changes to the function of the HPA axis that persist into young adulthood.

In this study there was no evidence of an association between exposure to childhood maltreatment and high inflammation levels as documented in other clinical cohorts such as the Dunedin Multidisciplinary Health and Development Study (DMHDS; Danese et al., 2008, 2007). Possible reasons for this could include the fact that individuals in the DMHDS were older (32 years of age). The pathophysiological process of establishing persistently high levels of systemic inflammation may require a lengthier period of time between insult and observation. Furthermore, the definition of child maltreatment in the DMHDS was broader, encompassing indices of maternal rejection, ratings of mothers' interaction with the index child, harsh discipline, and disruptive caregiver changes, in addition to reports of sexual and physical abuse. It is possible that these differences may account for the lack of evidence in our sample of an association between childhood maltreatment and clinically high levels of inflammation.

The fact that no link between early life adversities and markers of the metabolic syndrome were observed could be attributed to differences in the nature of sample and measurement methods of the present study compared to those of published studies that do report such an association. For example, the studies conducted by Barker and colleagues (D Barker, Gluckman, et al., 1993; D Barker, Hales, et al., 1993) demonstrating an association between low birth weight and dyslipidaemia and impaired glucose tolerance, comprised adults who were in their late stages of adulthood. It is likely that these documented associations could be a consequence of enduring sequelae that built up over the course of adulthood, which may not have been detectable in the mid-twenties, the age of the participants in the present study. Furthermore, in the retrospective study carried out by Entringer and colleagues (2008) into the effects of prenatal maternal stress on offspring glucose metabolism, an effect was observed for the glucose tolerance test but no differences were observed for plasma glucose levels. This suggests that effect sizes are small and require a higher level of power for differences to be detected.

Studies that have documented an association between childhood maltreatment and metabolic parameters have reported effects of physical and sexual abuse on BMI, but not other forms of abuse or neglect (Irish et al., 2010; Norman et al., 2012; Williamson et al., 2002). Moreover, an association between childhood maltreatment and hyperglycaemia was observed amongst middle-aged adults (Midei et al., 2013; Thomas et al., 2008). It is possible that in the present study the wider definition of maltreatment that encompassed physical, sexual, and emotional abuse as well as neglect, in conjunction with a younger age at the time of sampling could account for the lack of findings in the present study.

4.5. Methodological limitations and directions for future research

Alongside the numerous strengths of this study, such as the use of a prospective design starting in pregnancy through 26 years to chart offspring development, and the collection of data through one-to-one interviews from which clinical diagnoses of DSM-IV diagnoses were rated, there are limitations that need to be highlighted.

First, the use of a longitudinal birth cohort study design meant that the group sizes for the variables of interest could not be selected artificially as would have been done in an experimental design. As a consequence, it is plausible that some of the results are skewed by uneven sample sizes. Whilst, statistical techniques were applied to correct for these differences, this came at the expense of reduced power, thereby increasing the likelihood of type II error. Furthermore, it has frequently been observed that risk factors often aggregate. This means that it was difficult to separate out the effects of particular risks, for example different abuse types when examining the effect of childhood maltreatment, as the group sizes would have become too small to yield any meaningful results. The converse of this phenomenon is that the study has strong ecological validity and illuminates natural risk trajectories, but also means that there is the risk of residual confounding from factors that have gone unmeasured. However, in an attempt to control for this, as many potential associated risks were included in initial univariate analyses.

A second limitation is the relatively small sample size. Given that a wide range of influences spanning over 25 years were investigated, a sample size of just over one hundred for the psychopathology analyses, and circa seventy for the physiological analyses, means that it was not possible to discern all potential risk influences. For example, in the biological reactivity analyses, the ideal analysis strategy would be to separate offspring groups based on the three main factors of interest, prenatal maternal depression, offspring childhood maltreatment and adulthood depression. However, this resulted in group sizes of miniscule numbers. The effects of

prenatal maternal depression and offspring childhood maltreatment were thus conducted separately. The small sample size also meant that analyses were not separated by gender, which could be a moderating factor for some effects.

Third, the SLCDS is drawn from an urban, predominantly working class population of families of White ethnic origin. Thus, these results may not be representative of the wider UK population. Indeed, this limitation is linked to the fact that psychosocial vulnerabilities for depression, such as the impact of a problem partner interaction, lack of support, and low self-esteem, in line with Brown and colleagues' (G. W. Brown & Harris, 1986; G. W. Brown & Prudo, 1981) stress/vulnerability model, were not directly tested in this thesis. Given the nature of the sample, the decision was made to investigate the impact of gross psychosocial adversities, such as experiences of severe abuse and neglect.

Fourth, the majority of mothers were diagnosed with ICD-9 neurotic depression during pregnancy. It is worth noting that this diagnoses states that along with depressed mood, anxiety is frequently present in this categorisation, and that mixed states of depression and anxiety are classified as "neurotic depression" (World Health Organization, 1978). Whilst maternal anxiety during pregnancy was explicitly measured using the Leeds Anxiety Scale, and accounted for in analyses, it is still possible that some of the observed foetal programming effects can be attributed to mixed states of maternal depression and anxiety during pregnancy.

Fifth, participants varied in the length of time between interview and returning their saliva samples. It was also not possible to be sure how closely participants adhered to the time schedule for the collection of their samples. There was a high use of medication with anti-inflammatory effect, with a large proportion of girls using hormonal contraception. Given the high proportion of medication use, exclusion of these participants would have considerably reduced the sample size. For future analyses it may be better to exclude participants with

medication use that may be particularly problematic to cortisol levels (e.g. anti-inflammatories, asthma inhalers, cortisone cream). Nevertheless, there were no significant statistical outliers in raw cortisol values among those who used medication in my reported significant findings.

Sixth, information about offspring lifestyle factors such as consumption of fast food, smoking, drinking and exercise habits were collected through a schedule devised for the study asking participants to provide personal weekly estimates. In hindsight, the study would have benefited from a more formal quantification of these behaviours based on concrete examples for each day of the past week. This would have increased reliability and validity of these measures.

Seventh, the fact that assessment of antisocial psychopathology in adulthood consists of generating Axis II personality disorders means that I was not able to extend earlier findings at 16 years in this domain. Given that only Axis I disorders was assessed at 25 years the findings relate only to depressive disorders. Indeed, at 16 we found that maltreatment moderated the effects of prenatal depression on offspring psychopathology, with this effect being strongest for antisocial psychopathology, namely for offspring with a conduct disorder diagnosis.

Finally, it would have been useful to take into account possible genetic contributions to offspring vulnerability to depression and physiological characteristics. This data would have helped to distinguish between observed programming effects and possible genetic predispositions.

Future research would benefit from examining psychosocial and biological mechanisms linking prenatal maternal depression to offspring child maltreatment. Identification of such pathways would provide the opportunity for the provision of preventive interventions. Furthermore, the role of fathers in the association between prenatal maternal depression, offspring childhood maltreatment and offspring depression would cast light on other psychosocial mechanisms at play. Also, given the high comorbidity between depressive and anxiety disorders, studies should

examine the impact of prenatal maternal stress on the development of each, when controlling for the other. This would illuminate whether prenatal maternal stress represents a risk specifically for offspring adulthood depression or for a more generalised vulnerability for internalising psychopathology.

To cast light further on the neurobiological mechanisms underpinning the link between prenatal maternal depression and offspring psychopathology, longitudinal studies should measure GCs, inflammatory markers and metabolic parameters in pregnant women in addition to psychological measures of stress. Research would also benefit from discriminating the neurobiological effects of prenatal maternal depression from childhood maltreatment, and also from considering the cumulative effects of both. This would provide important theoretical information on the putative biological mechanisms underpinning the link between early life adversity and stress reactivity and cardio-metabolic dysfunction.

Future studies should also consider a wider range of markers of inflammation including a battery of cytokines, chemokines and adhesion molecules. It would also be worth investigating the epigenetic changes in gene expression particularly for genes relating to the GR receptor. Animal studies have shown down-regulation of candidate genes for the GR as a function of exposure to maternal depression *in utero*. Human evidence for this mechanism would provide greater insight into the function mechanisms underpinning HPA axis dysregulation in offspring exposed to prenatal maternal stress.

Finally, neuroimaging studies into the functional and structural changes that may occur as a consequence of exposure to prenatal maternal stress may provide biological correlates for the commonly observed changes in psychological function in offspring exposed to prenatal maternal stress.

4.6. Clinical implications

The findings of this thesis have significant implications for policy and clinical practice. Until recently, UK policy primarily advocated the screening of maternal depression during the postnatal period. In the last few years there has been a shift in focus to the prenatal period. Indeed, a recently released UK cross-party manifesto, "1001 Critical Days: The Importance of the Conception to Age Two Period. A Cross-Party Manifesto" (Leadsome, Field, Burstow, & Lucas, 2013) advocates the importance of addressing maternal mental health issues during pregnancy to ensure the healthy development of the child. Previously published findings from the SLCDS have contributed to this manifesto, and results from the current 25-year phase provide clear evidence for the long-term detrimental effects of untreated maternal depression specifically during pregnancy.

My findings demonstrate that untreated prenatal maternal depression predicts offspring child maltreatment and depression during young adulthood, with ramifications for offspring 25 years after mothers were first identified as depressed. By intervening during pregnancy, the prevalence of child maltreatment could be reduced, as well as rates of affective disorders in the young adult population. Targeting prenatal maternal depression during pregnancy could increase the wellbeing of our offspring as well as reduce the financial burden on the NHS due to the high prevalence of depression amongst our youth. All pregnant women should be screened for depression early on during their pregnancy and offered support and monitoring from professionals such as midwives and general practitioners, as well as being prioritised for access to psychological therapies for the treatment of depressive episodes.

4.7. Conclusions

In summary, several novel findings emanate from the data of this PhD thesis. Offspring exposed to maternal depression during gestation were found to experience significantly more episodes of clinically diagnosed depression during young adulthood. Key pathways comprised an increased risk of exposure to maltreatment, as well as further exposure to maternal depression during childhood, both of which contributed additively to the heighted risk of suffering depression as a young adult. Furthermore, offspring exposed to prenatal maternal depression were observed to show elevated levels of systemic inflammation, whilst offspring exposed to childhood maltreatment exhibited abnormalities in their HPA axis activity. Dysregulation in both of these systems has been shown to be involved in the pathogenesis of depression. This observed biological dysfunction likely represents biological embedding of psychosocial stress into risk for depressive psychopathology. Overall, these findings demonstrate that exposure to prenatal maternal depression has persistent consequences on psychological development as well as neurobiological function.

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Appendices

Appendix A: Information sheet

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Participant Information Sheet – Young Adult

Study Title
What are the young adult health outcomes for the children of the South London Child Development Study?

Invitation paragraph
We would like to invite you to participate in the eighth phase of the South London Child Development Study, which you and your family have been a part of since before your birth. We remain greatly appreciative of your willingness to participate in previous stages of our research study. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?
We have been interested in investigating your personal development, social and family life, cognitive development and emotional well being from your birth until adolescence. We are now interested in examining your adulthood development.

Why have I been invited?
You have been invited to participate as you have taken part in at least one of the previous study sessions of the South London Child Development Study, and we would like to visit you now that you are an adult.

Do I have to take part?
No, it is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?
Participation in this phase of the study will be similar to previous stages that you have taken part in. We will invite you to visit us, or request your permission to visit you in your home for a 2-3 hour interview session. We will also arrange a visit with your mother/parental figure, but that will take place at a different time. During the visit we will interview you about your emotional well-being, your relationship with your mother/parental figure, your social and occupational life and assess your cognitive abilities. We will also measure your weight, height, waist circumference and stress levels by taking a blood sample and saliva sample.
The interview will be conducted by a trained researcher and will be scheduled for a time that is convenient to you. During the interview we will use a mixture of questionnaires and interviews. You will not have to answer any questions which make you feel uncomfortable, and you will be able to stop the interview whenever you wish. We will ask for your permission to audio-record the interviews, and these recordings will be deleted once we have transcribed them. We will also take a blood sample to look at hormone and protein levels and DNA for genetic studies (30mls blood – about 2 tablespoons) and a saliva sample to look at proteins relevant to inflammation. You will be asked to provide 6 specimens of your saliva on one day during the week after our visit. You will be shown how to do this during the assessment visit. We are looking at cortisol ("stress hormone") levels in saliva.
Everything that you tell us during the assessment session will remain confidential between yourself and us. The only exception would be if there was something that would pose a danger to yourself or someone else, in which case we may need to contact another professional body such as your GP

Expenses and payments
You will receive £50 for participating, plus we will reimburse your travel expenses.

What will I have to do?
If you wish to take part in the study, you will be asked to sign the consent form at the end of this document; you will be given a copy to keep. You should be prepared to undertake the study visits, as detailed above, in the research lab or in your own home. If you have recent or current participation in other research studies please consider whether you should also participate in this study.

What are the possible disadvantages and risks of taking part?
You may experience some minor discomfort and/or bruising from the blood sample.

During the study, it is possible that other conditions are discovered of which you were unaware, which may have implications for your future health, or otherwise impacts on your interests. If anything is identified, your GP will be informed, with your agreement.

What are the possible benefits of taking part?
There are no direct benefits to you of taking part in the study; however the knowledge gained from this study may be of help to other people in the future.

What do I do if I want to withdraw from the study?
You are free to withdraw from the study at any time you like. You will not be required to give us any reasons for withdrawal.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed; detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence; details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision

Part 2

What will happen if I don't want to carry on with the study?
Should you wish to stop participating in the study, in addition to withdrawing yourself from the study, you have the right to withdraw any data/information you have provided up until it is included in final report that have been submitted for publication. We will start to write-up our findings within the year after completing the study, so you can request your data be withdrawn within this time period. After publication we will not be able to withdraw your data from the published articles, but we will still be able to withdraw it from the study database to prevent its future use.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (tel. 0207 848 0353). If you remain unhappy & wish to complain formally, you can do this through the King's College Complaints Procedure; details can be obtained from the college.

Will my taking part in the study be kept confidential?
Yes, your confidentiality will be safeguarded during and after the study, which is conducted in accordance with the Data Protection Act 1998. The same identification code used at previous study phases will be allocated to you. We will use this ID code to label your questionnaires, interviews and biological samples, and your personal details will not be linked to the information you give us, nor will they be shared with anyone outside of the research team. The information we collect will be recorded and put into electronic databases using this code rather than your name. Paper and electronic records are stored securely to which only the study researchers will have access. The custodian of all study materials is Dr Susan Pawlby (Chief Investigator).

Study data will be analysed and results will be submitted for publication; your identity will not be revealed. Study data will be retained and may be used in future studies, if this happens, further Research Ethics Committee approval will be sought. Authorised persons such as researchers, sponsors, regulatory authorities and Research and Development audit will have access to view identifiable data, for monitoring of the quality of the research. Study data will be retained for 10 years after completion of the study; and will be disposed of securely. You have the right to check the accuracy of data held about you and correct any errors according to local law and procedures.

What will happen to any samples I give?
All samples will be processed then stored prior to analysis using the identification code already described. The researchers and laboratory scientists will have access to the samples, and the researchers will link your other study data to data from the analysis of your sample by the identification code. Blood and saliva samples will be kept for three years.

Will any genetic tests be done?
Yes, we will look at genetic material (DNA) which might be relevant to the development of stress, inflammation, emotional and behavioural problems.

What will happen to the results of the research study?

The data and results from this study may be published in medical journals or used in scientific reports and may be communicated to the regulatory authorities. You will not be identified by name. Once the study has been completed, a report of the findings will be prepared for participants and will be posted to you upon completion of the study.

Who is organising the research study?
The chief investigator, Dr Susan Pawlby, is organising the research, which is sponsored by the Institute of Psychiatry, King's College London. The study is funded by the Psychiatry Research Trust charity. This phase of the study is being carried out as PhD project**.** The researchers involved in conducting this study to not receive any financial incentives for including you in this study and do not benefit financially from this study.

Who has reviewed the study?
This research has been looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the London – Camberwell St Giles Research Ethics Committee.

Further information and contact details

Primary contact: Dominic Plant (02078480353 or 07574682067 or dominic.plant@kcl.ac.uk)

Secondary contact: Dr Susan Pawlby (02078480353 or susan.pawlby@kcl.ac.uk)

Thank you for reading this information sheet.

Appendix B: Consent form

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Consent Form

Study Title: What are the young adult health outcomes for the children of the South London Child Development Study?

Participant Identification Number:	Initial
I confirm that I have read & understood the participant information sheet dated 05.01.12 for the above study. I have had the opportunity to consider the information, ask questions & have had these answered satisfactorily.	
I understand that my participation is voluntary & that I am free to withdraw at any time without giving any reason and without medical care or legal rights being affected. Furthermore, I understand that I will be able to withdraw my data up until publication of the study findings.	
I agree to be contacted in the future by King's College London researchers who would like to invite me to participate in follow up studies to this project, or in future studies of a similar nature.	
I understand that information held by the NHS and maintained by the Information Centre may be used to keep in touch with me	
I agree to give either a sample of blood or cheek swab, and saliva samples for research in the above project. I understand how the sample will be collected, that giving the sample is voluntary and that I am free to withdraw at any time without giving a reason, and without my medical treatment or legal rights being affected. I understand that the blood and saliva samples will be kept for the duration of the project (3 years)	
I understand that research using the sample I give will involve genetic analysis aimed at understanding the role of genes in stress and health, that the data produced are for research rather than clinical purposes, and that these results will have no implications for me personally	
I understand I will not receive any 'test' results from this study, because the assessment I will undergo, does not produce clinically relevant information but just research data. The project newsletter will describe the general importance of any research results obtained.	
I agree that the research team may use my data for future research and understand that any such use of identifiable data would be reviewed and approved by a research ethics committee. (In such cases, as with this project, data would not be identifiable in any report).	
I understand that the information I disclose will be treated as strictly confidential, with the exception of something that may pose a danger to myself or others, in which case a relevant third party such as my GP may be contacted.	
I agree to take part in the above study.	

Name of participant: _____

Signature of participant: _____ Date:

Name of researcher: _____

Signature of researcher:_____ Date:

Appendix C: Personal characteristics schedule

South London Child Development Study YOUNG PERSON INTERVIEW

Date of interview *dd/mm/yy*	……/……../……	**Young person ID**				

Section A: Background Information

Questions should be completed prior to the interview and clarified with interviewee

1	**Interviewer's name** *(code)* **(DP=1; SP = 2; JW = 3)**	
2	**Young person's full name:** **Previous surname if different:**	
3	**Address:**	
4	**Gender:** M F	
5	**Date of birth:**	……../……../……..
6	**Telephone:**	

Codes:

-8 if not answered/not applicable
-9 if missing

I am now going to ask you about your living circumstances and current relationships.

Do you live alone? Who else lives with you? (If you are a student who has a term-time and fixed home address please describe your home/family residence)

8a	**No = 0 Yes = 1**			
	i) Name (in full)	**ii) Sex**	**iii) Relationship to you (use codes below)**	**iv) Age (DOB if offspring)**
b				
c				
d				
e				
f				
g				

	Code	**Relationship**
	1	Biological mother
	2	Biological father
	3	Stepmother
	4	Stepfather
	5	Foster/adoptive mother
	6	Foster/adoptive father
	7	Biological sibling
	8	Half brother/half sister
	9	Biological child (offspring)
	10	Adoptive child
	11	Foster sibling
	12	Grandparent
	13	Partner
	14	Other

9	**What type of housing are you living in now?** *(Circle one code)*			
	Owner/occupier	1	Rented from local authority	5
	Privately rented	2	Temporary accommodation	6
	Shared ownership (part buy/rent)	3	Living with parents/family home	7
	Rented from housing association	4	na	-8

10	**How would you describe your current marital status?**	**Code (circle)**
	Single	0
	Married	1
	Partner (co-habiting)	2
	Partner (not-cohabiting)	3
	Widowed: single	4
	Widowed: remarried	5
	Widowed: cohabiting	6
	Divorced: single	7
	Divorced: remarried	8
	Divorced: cohabiting	9

	Separated: single	10
	Separated: remarried	11
	Separated: cohabiting	12
	Other ……..	13
	n/a	-8
11	**If not single - How long have you been with your current partner?**	**Code (circle)**
	< 1 year	0
	1-2 years	1
	> 2 years	2
	N/a	-8

12a	**Do you have a child that doesn't live with you?**	**Code (circle)**		
	No	0		
	Yes	1		
b	Yes: lives with partner	1		
	Yes: lives with relatives	2		
	Yes: state care	3		
	Yes: other [state] ……..	4		
	N/A	-8		
13i	**Do you have a child; if so how many?**	**Code (circle)**		
	No	0		
	Yes	1		
ii	Enter number			
	N/A	-8		
14	**Is your current partner the father/mother of x?**	**Code (circle)**		
	No	0	0	0
	Yes	1	1	1
	N/A	-8	-8	-8
19	**Does your partner work?**	**Code (circle)**		
	NO	0		
	Yes, part time (<20 hrs/week)	1		
	Yes, full time	2		
	Studying	3		
	Other ……..	4		
	N/A	-8		

20	Where was your partner born?	Code (circle)
	UK	0
	Europe	1
	Ireland	2
	West Indies	3
	Africa	4
	Bangladesh	5
	Pakistan	6
	India	7
	Cyprus, Turkey, Greece	8
	New Zealand, Australia, Canada, USA, SA	9
	Other [state] …….	10
	N/A	-8

21	How would you describe your partner's ethnicity?		Code (circle)
	White British		0
	White Other		1
	Black:	Black British (born in UK)	2
		Black British / Caribbean	3
		Black British / African	4
	Mixed:	Mixed White & Black Caribbean	5
		Mixed White & Black African	6
		Mixed White & Asian	7
	Asian:	Asian - Indian	8
		Asian - Pakistani	9
		Asian - Bangladeshi	10
	Chinese		11
	Other [state] ……		12
	n/a		-8

22	How old was your partner when X was born?	Code (1 column/child)		
		22a	22b	22c
	Enter age			
	n/a	-8	-8	-8
23	How old where you when you had X?	23a	23b	23c
	Enter age			
	n/a	-8	-8	-8
24a	Have you had any previous pregnancies?			
	No	0	0	0
	Yes	1	1	1
	Na	-8	-8	-8
24b	If yes, how old were you?			
	(Record age)			
24c	If yes, what was the outcome?			
	Termination	1	1	1

	Miscarriage	2	2	2
	Still birth	3	3	3
	Other ……..	4	4	4
	Na	-8	-8	-8

Education, Employment & Earnings

In this section I'd like to know about your education history and current employment and/or student activities.

25	**Please list ALL qualifications , INCLUDING details of course, university etc**			
	No qualifications	1		
	Some GCSEs	2		
	A Levels	3		
	BTech / NVQ levels 1-3 (apprenticeship)	4		
	Professional Qualification without degree (e.g. HND, SRN)	5		
	University degree	6		
	Post bachelors (i.e. Masters/PhD)	7		
	Other …….	8		
	na	-8		
26a	**Are you currently working?**	**Code (circle)**		
	No	0		
	Yes	1		
b	Ft (1) or PT (2)	1/ 2		
	na	-8		
27	**When was your first job? (include temporary work/holiday etc)**	**Code (circle)**		
	<16 (i.e. during school)	1		
	16-17	2		
	18-19	3		
	20-21	4		
	22-23	5		
	24-25	6		
	>25	7		
	Never worked	8		
	Na	-8		
28a	**Have you had any period of unemployment when you were not studying or travelling? (total number of months). How long?**	**Code (circle)**		
	No; Always either employed/studying/travelling	0		
	Yes	1		
b	<6 months	1		
	6-12 months	2		
	12-18 months	3		
	18-24 months	4		
	<24 months	5		
	na	-8		
29a	**Have you ever been dismissed from a job?**	**Code (circle)**		
	No	0	0	0
	Yes	1	1	1
29b	Yes: minor misconduct [state] ……	1	1	1
	Yes: gross misconduct [state] …….	2	2	2

	Resignation [state] …….	3	3	3
	Redundancy	4	4	4
	na	-8	-8	-8
30a	**Are you currently receiving any of the following government benefits?**	**Code (-8 if na)**		
	No	0		
	Yes	1		
30b	Council tax benefit	1		
	Child benefit	2		
	Tax credit	3		
	Invalidity benefit	4		
	Housing benefit	5		
	Job seekers allowance	6		
	Income support	7		
	Disability living allowance	8		
	Incapacity benefit	9		
	Hardship fund	10		
31	**What is your approximate household annual income (include partner's if living with partner or parents) GROSS, pre-tax**	**Code**		
	Up to £10,000	1		
	£11,000 - £20,000	2		
	£21,000 – £30,000	3		
	£31,000 – £40,000	4		
	£41,000 – £50,000	5		
	£51,000 - £60,000	6		
	£61,000 - £70,000	7		
	£71,000+	8		
	na	-8		
32	**Do you have financial problems that you consider unmanageable?**	**Code**		
	No	0		
	A few	1		
	A lot	2		
	n/a	-8		

Appendix D: Lifestyle and health schedule

Health Behaviours

35a	**Do you exercise regularly**	**Code**
	No	0
	Yes	1
	Na	-8
35b	**If yes, how often per week on average?**	
	Less than once a week	1
	1-2 times per week	2
	3-4 times per week	3
	Almost everyday (5-7)	4
	NA	-8
35c	**What kind of exercises do you do? (code all)**	
	Run/jog	1
	Weights	2
	Swim	3
	Walk	4
	Row	5
	Cycle	6
	Other	7
	Na	-8
36	**How often do you eat take-out/ready meals per week**	
	Less than once per week	0
	1-2 times per week	1
	3-4 times per week	2
	Almost every day (5-7)	3
	More than once a day	4
	N/A	-8
37a	**Do you currently smoke?**	
	No	0
	Yes	1
	N/A	-8
37b	**If yes, on average how many per day?**	
	<5	1
	>6<10	2
	>11<15	3
	>16<20	4
	>21	5
37c	**Did you used to smoke?**	
	No	0
	Yes	1
	N/A	
37d	**Previous smoking duration? (code if 1 month or more)**	
	< 1 year	1

	1-2 years	2
	> 2 years	3
	NA	-8
38a	**Do you drink?**	
	No	0
	Yes	1
	Na	2
38b	**If yes, on average how many nights per week?**	
	Less than once per week	1
	1-2 times per week	2
	3-4 times per week	3
	Almost every day (5-7)	4
	N/A	-8

CURRENT MEDICATION

34. Are you currently taking any medications, including oral medication (including dietary supplements), creams and inhalers?

0 = No 1 = Yes

a) Drug Name_____

i) Dose (mg/day) _____

ii) Start date *(month / year)* _____ / _____

iii) Route of administration: _____

b) 0 = No 1 = Yes

Name_____

i) Dose (mg/day) _____

ii) Start date *(month / year)* _____ / _____

iii) Route of administration: _____

c) 0 = No 1 = Yes

Name_____

i) Dose (mg/day) _____

ii) Start date *(month / year)* _____ / _____

iii) Route of administration: _____

d) Record any other drugs here in the same way

_____

Appendix E: Life events schedule

Life Events

7) *I'd like to start by finding out about any important events or changes in your life since you were 16 years old. It's important that you try to think back over the whole 9 year period. I'll give you some examples of the king's of things I'm thinking of.*

Code: 0 = Not at all distressing
1 = Mildly distressing
2 = Moderately distressing
3 = Severely distressing

Event (1 column/event)	**Occurred (no/yes) 0 = no; 1 = yes; don't**		**If yes, age (Yrs-mths) AND dates**		**Details of event**		**Distress (1 column/ event)**	
	Ai)	**Bi)**					**Aii)**	**Bii)**
7a) A serious illness or injury to yourself								
b) A serious illness or injury to a close relative								
c) The death of a close relative								
d) The death of a close friend								
e) Separation from your spouse due to marital distress								
f) Broken off a steady relationship								
g) A serious problem with a close friend, neighbour or relative								

h) Been unemployed/ seeking work for more than 1 month								
i) Been sacked from your job/changed jobs								
j) Had a change of accommodation								
k) Had a major financial crisis								
l) Had something valuable lost or stolen								
m) Other please specify								
EXTRAS: (enter number)								

Appendix F: Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV) – mood disorders section

STRUCTURED CLINICAL INTERVIEW

FOR DSM-IV AXIS I DISORDERS

A. MOOD EPISODES

CURRENT MAJOR DEPRESSIVE EPISODE CRITERIA

Now I am going to ask you some more questions about your mood.	(A) Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.	
A1 In the past month . . . … has there been a period of time when you were feeling depressed or down most of the day nearly every day? (What was that like?) IF YES: How long did it last? (As long as 2 weeks?)	(1) depressed mood most of the day, nearly every day as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g. appears tearful).	**A1 current ? - +**
A2 . . . what about losing interest or pleasure in things you usually enjoyed? IF YES: Was it nearly every day? How long did it last? (As long as 2 weeks?)	(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).	**A2 current ? - +**

If **neither A1 nor A2** is "+" during the current month, check for past Major Depressive episode by asking questions A1 and A2 again looking for lifetime (since last assessment) episodes, beginning with "Has there EVER since we last visited you (11 or 16)…"

IF AT LEAST ONE PAST DEPRESSED PERIOD: Have you had more than one time like that? Which one was the worst?

FOR THE FOLLOWING QUESTIONS, FOCUS ON THE WORST TWO WEEKS OF THE LAST MONTH (OR PAST TWO WEEKS IF EQUALLY DEPRESSED FOR THE ENTIRE MONTH) OR SINCE LAST VISIT IF NO CURRENT SYMPTOMS

During [2-WEEK PERIOD] … From: …../…../…… To: …./…../…… *(insert dates)*

A3 . . .did you lose or gain any weight? (How much? Were you trying to lose weight?) IF NO: How was your appetite? (What about compared with your usual appetite?) Did you have to force yourself to eat? Eat [less/more] than usual? Was that nearly every day?)	(3) significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.	**A3** **? - +**
A4 . . how were you sleeping? (Trouble falling, waking frequently, trouble staying asleep, waking too early, OR sleeping too much? How many hours a night compared with usual? Was that nearly every night?)	(4) insomnia or hypersomnia nearly every day	**A4** **? - +**

A5 . . were you so fidgety or restless that you were unable to sit still? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?) IF NO: What about the opposite - talking or moving more slowly than is normal for you? (Was it so bad that other people noticed it? What did they notice? Was that nearly every day?)	(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down) *NOTE:* ALSO CONSIDER BEHAVIOUR DURING THE INTERVIEW	**A5** **? - +**
A6 . . what was your energy like? (Tired all the time? Nearly every day?)	(6) fatigue or loss of energy nearly every day	**A6** **? - +**
A7 . . how did you feel about yourself? (Worthless? Nearly every day?) IF NO: What about feeling guilty about things you had done or not done? (Nearly every day?)	(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick) *NOTE:* CODE "-" IF ONLY LOW SELF-ESTEEM	**A7** **? - +**
A8 . . did you have trouble thinking or concentrating? (What kinds of things did it interfere with? Nearly every day?) IF NO: Was it hard to make decisions about everyday things?	(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)	**A8** **? - +**

A9 . . were things so bad that you were thinking a lot about death or that you would be better off dead? What about thinking of hurting yourself? IF YES: Did you do anything to hurt yourself?	(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide	**A9** **? - +**

A10 **AT LEAST FIVE OF A1 TO A9 ARE "+" AND AT LEAST ONE OF THESE IS ITEM A1 OR A2**	**A10** **? - +**

If **A10** above is "-" (i.e. fewer than five are "+") ask the following if unknown: Has there been any <u>other</u> times since we last saw you, when you've been depressed and had even more of the symptoms that we've just talked about?

A11 IF UNCLEAR: Has (the depression/OWN WORDS) made it hard for you to do your work, take care of things at home, or get along with other people?	C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.	**A11** **? - +**

If **A11** above is "-" (i.e. symptoms not clinically significant) ask the following if unknown: Has there been any <u>other</u> times since we last saw you when you've been depressed and it had more of an effect on your life?

A12 Just before this began, were you physically ill? Just before this began, were you taking any medications? IF YES: Any change in the amount you were taking? Just before this began, were you	D. The symptoms are not due to the direct effects of a substance (e.g. a drug of abuse, medication) or to a general medical condition. <u>Etiological general medical conditions include</u>: degenerative neurological illnesses (e.g., Parkinson's disease), cerebrovascular disease (e.g., stroke), metabolic conditions (e.g., Vitamin B-12 deficiency), endocrine conditions (e.g., hyper- and hypothyroidism, hyper- and hypoadreno-corticism); viral or other infections (e.g., hepatitis,	**A12** **? - +**

drinking or using any street drugs?	mononucleosis, HIV), and certain cancers (e.g., carcinoma of the pancreas). Etiological substances include: alcohol, amphetamines, cocaine, hallucinogens, inhalants, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include antihypertensives, oral contraceptives, corticosteroids, anabolic steroids, anticancer agents, analgesics, anticholinergics, cardiac medications.	

If **A12** above is "-" (i.e. mood is due to substance or general medical condition), ask:

Have there been any other times since we last saw you when you've been depressed and it was not because of [GENERAL MEDICAL CONDITION/ SUBSTANCE USE]?

A13 IF UNKNOWN: Did this begin soon after someone close to you died?	E. The symptoms are not better accounted for by Bereavement, i.e., after the loss [death] of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.	**A13** **? - +**

If **A13** above is "-" (i.e. the depressed mood is better accounted for by Bereavement), ask: Have there been any other times since we last saw you when you've been depressed and it was not because of the loss of a loved one?

A14 CRITERIA A, C, D & E ARE "+"	**MAJOR DEPRESSIVE EPISODE**	**A14** **? - +**
A15 How many separate times have you been (depressed/ OWN WORDS) nearly every day for at least 2 weeks and had several of the symptoms that you described, such as (SYMPTOMS)?	Total number of Major Depressive Episodes including current. (CODE 99 if too numerous or indistinct to count). _____	**A15**

MANIC EPISODE CRITERIA

A16 Have you ever had a period of time when you were feeling so good, high, excited, or hyper that other people thought you were not your normal self or you got into trouble? (Did anyone say you were manic? Was that more than just feeling good?) What was that like? IF NO: What about a period of time when you were so irritable that you found yourself shouting at people or starting fights or arguments? (Did you find yourself yelling at people you didn't really know?)	A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood . . .	**A16** **? - +**

If **A16** is "-" (i.e. never any periods of elevated or irritable mood) go to **A45** (*Dysthymic Disorder*)

A17 How long did that last? (As long as one week?) (Did you have to go into a hospital?)	. . . lasting at least 1 week (or any duration if hospitalization is necessary)	**A17** **? - +**

If **A17** is "-" (i.e. duration is less than 1 week) go to **A30** (*Hypomanic Episode*)

Have you had more than one time such as that? Which time were the most [high/irritable/OWN WORDS]?

FOR ITEMS **A18 – A27** FOCUS ON THE MOST EXTREME EPISODE.

IF UNKNOWN: During this time, when were the most [OWN WORDS for euphoria or irritability]?

During (PERIOD OF WORST MANIC SYMPTOMS) …	B. During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if mood is only irritable) and have been present to a significant degree:

A18 . . .how did you feel about yourself? (More self-confident than usual? Any special powers or abilities?)	(1) inflated self-esteem or grandiosity	**A18 ? - +**
A19 . . .did you need less sleep than usual IF YES: Did you still feel rested?	(2) decreased need for sleep (e.g. feels rested after only 3 hours of sleep)	**A19 ? - +**
A20 . . .were you much more talkative than usual? (Did people have trouble stopping you or understanding you? Did people have trouble getting a word in edgewise?)	(3) more talkative than usual or pressure to keep talking	**A20 ? - +**
A21. . .were your thoughts racing through your head?	(4) flight of ideas or subjective experience that thoughts are racing	**A21 ? - +**
A22 . . .were you so easily distracted by things around you that you had trouble concentrating or staying on one track?	(5) distractibility (i.e. attention too easily drawn to unimportant or irrelevant external stimuli)	**A22 ? - +**
A23. . .how did you spend your time? (Work, friends, hobbies? Were you so active that your friends or family were concerned about you?) IF NO INCREASED ACTIVITY: Were you physically restless? (How bad was it?)	(6) increase in goal- directed activity (socially, at work or school, or sexually) or psychomotor agitation	**A23 ? - +**
A24. . did you do anything that could have caused trouble for you or your family? (Buying things you didn't need? Anything sexual that was unusual for you? Reckless driving?)	(7) excessive involvement in pleasurable activities which have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments)	**A24 ? - +**
A25 **AT LEAST THREE OF B1 – B7 ARE "+" (OR FOUR IF MOOD IRRITABLE AND NOT ELEVATED)**		**A25 ? - +**

If **A25** above is "-" (i.e. fewer than three are "+") ask the following:

Have there been any other times since we last saw you, when you were [high/irritable/OWN WORDS]

and had even more of the symptoms that we've just talked about?

A26 IF NOT KNOWN: At that time, did you have serious problems at home or at work (school) because you were [SYMPTOMS] or did you have to go into a hospital?	D. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.	**A26 ? - +**

If **A26** above is "-" (i.e. not sufficiently severe) ask the following:

Have there been any other times since we last saw you when you were [high/irritable/OWN WORDS]

and you got into trouble with people or were hospitalized?

A27 Just before this began, were you physically ill? Just before this began, were you taking any medications? IF YES: Any change in the amount you were taking? Just before this began, were you drinking or using any street drugs?	E. The symptoms are not due to the direct physiological effects of a substance (e.g a drug of abuse, medication) or to a general medical condition. **Note:** Manic-like episodes that are clearly caused by somatic antidepressant treatment (e.g. medication, electroconvulsive therapy, light therapy) should not count toward a diagnosis of Bipolar I Disorder but are considered Substance-Induced Mood Disorders. <u>Etiological general medical conditions include</u> degenerative neurological illnesses (e.g., Huntingdon's disease, multiple sclerosis), brovascular disease (e.g., stroke), abolic conditions (e.g., Vitamin B-12 iency, Wilson's disease), endocrine itions (e.g. hyperthyroidism), viral or other tions and certain cancers (e.g. cerebral blasms). <u>Etiological substances include:</u> alcohol, amphetamines, cocaine, hallucinogens, inhalants, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include psychotropic medications (e.g.	**A27 ? - +**

If there is any indication that the mania may be secondary (i.e., a direct physiological consequence of a general medical condition

	antidepressants), corticosteroids, anabolic steroids, isoniazid, antiparkinson medication (e.g. levodopa), and sympathominimetics/ decongestants.	

A28 CRITERIA A, B, D AND E ARE " +" **MANIC EPISODE** IF UNKNOWN: Have you had (SYMPTOMS RATED "+" ABOVE) in the past month? Check here _____ if criteria have been met in the past month		**A28 +**
How many separate times since we last saw you were you [HIGH/OWN WORDS] and had [ACKNOWLEDGED MANIC SYMPTOMS] for at least a week (or were hospitalized)?	Total number of Manic Episodes, including current, (CODE 99 if too indistinct or too numerous to count) _____	**A29**

HYPOMANIC EPISODE CRITERIA

A30 IF UNKNOWN: When you were [HIGH / IRRITABLE / OWN WORDS], did it last for at least 4 days? Have you had more than one time like that? (Which time were you the most [high / irritable / OWN WORDS]?)	A. A distinct period of persistently elevated, expansive, or irritable mood, lasting throughout at least 4 days, that is clearly different from the usual non-depressed mood.	**A30 ? - +**

FOR ITEMS A31 – 37, FOCUS ON THE MOST EXTREME EPISODE

During [PERIOD OF MOST EXTREME HYPOMANIC SYMPTOMS] …	B. During the period of mood disturbance, three (or more) of the following symptoms have persisted(four if the mood is only irritable) and have been present to a significant degree:	
A31… how did you feel about yourself? (More self-confident than usual? Any special powers or abilities?)	(1) inflated self-esteem or grandiosity	**A31 ? - +**

A32 . . did you need less sleep than usual? IF YES: Did you still feel rested?	(2) decreased need for sleep (e.g., feels rested after only 3 hours of sleep)	**A32** **? -** **+**
A33 . . were you much more talkative than usual? (Did people have trouble stopping you or understanding you? Did people have trouble getting a word in edgewise?)	(3) more talkative than usual or pressure to keep talking	**A33** **? -** **+**
A34. . were your thoughts racing through your head?	(4) flight of ideas or subjective experience that thoughts are racing	**A34** **? -** **+**
A35 . . were you so easily distracted by things around you that you had trouble concentrating or staying on one track?	(5) distractibility (i.e. attention too easily drawn to unimportant or irrelevant external stimuli)	**A35** **? -** **+**
A36 . . how did you spend your time? (Work, friends, hobbies? Were you so active that your friends or family were concerned about you?) IF NO INCREASED ACTIVITY: Were you physically restless? (How bad was it?)	(6) increase in goal- directed activity (either socially, at work or school, or sexually) or psychomotor agitation	**A36** **? -** **+**
A37 . . did you do anything that could have caused trouble for you or your family? (Buying things you didn't need? Anything sexual that was unusual for you? Reckless driving?)	(7) excessive involvement in pleasurable activities which have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments)	**A37** **? -** **+**
AT LEAST THREE OF B1 – B7 ARE "+" (OR FOUR IF MOOD IS IRRITABLE AND NOT ELEVATED)		**A38** **? -** **+**

If **A38** is "-" (i.e. fewer than three are "+") ask the following:

Have there been any <u>other</u> times since we last saw you when you were [high/irritable/OWN WORDS] and had even more of the symptoms that we've just talked about?

A39 IF UNKNOWN: Is this very different from the way you usually are? (How	C. The episode is associated with an unequivocal change in functioning that is	**A39** **? -**

were you different? At work? With friends?)	uncharacteristic of the person when not symptomatic.	**+**

If **A39** is "-" (i.e. characteristically "hypomanic"), ask the following:

Have there been any <u>other</u> times since we last saw you when you were [high/irritable/OWN WORDS]

and you were really different from the way you usually are?

A40 IF UNKNOWN: Did other people notice the change in you? (What did they say?)	D. The disturbance in mood and the change in functioning are observable by others.	**A40 ? - +**

If **A40** is "-" (i.e. not observable by others) ask the following:

Have there been any <u>other</u> times since we last saw you when you were [high/irritable/OWN

WORDS] and other people <u>did</u> notice the change in the way you were acting?

A41 IF UNKNOWN: At that time, did you have serious problems at home or at work (school) because you were [SYMPTOMS] or did you have to go into a hospital?	E. The episode is not severe enough to cause marked impairment or to need hospitalization, and no psychotic features.	**A41 ? - +**

If **A41** is "-" (i.e. severe enough to cause marked impairment), go back to **A26,** code "+" for that item,

and continue with **A27**.

A42 Just before this began, were you physically ill? Just [illegible] any [illegible] med[illegible] IF YE[illegible] were taking? Just before this began, were you drinking or using any street drugs?	F. The symptoms are not due to the direct physiological effects of a substance (e.g a drug of abuse, medication) or to a general condition. [illegible]ypomanic-like symptoms that are [illegible]aused by somatic antidepressant [illegible]nt (e.g. medication, electroconvulsive therapy, light therapy) should not count toward a diagnosis of Bipolar II Disorder but are considered Substance-Induced Mood Episodes. *Refer to list of possibly etiological general medical conditions and substances included with item **A27**.*	**A42** **? - +**

If there is any indication that the hypomania may be secondary (i.e., a direct physiological consequence of a general medical condition or

If **A42** above is "-"(i.e. the hypomania is due to a substance or general medical condition), ask the following:

A43 **CRITERIA A, B, C, D, E AND F ARE " +" HYPOMANIC EPISODE** IF UNKNOWN: Have you had (SYMPTOMS RATED "+" ABOVE) in the past month? Check here _____ if criteria met in the past month		**A43** **+**
How many separate times since we last saw you were you (high/irritable/OWN WORDS) and had [ACKNOWLEDGED HYPOMANIC SYMPTOMS] for a period of time?	Total number of Hypomanic Episodes, (CODE 99 if too indistinct or numerous to count _____	**A44**

YOU ARE FINISHED EVALUATING MOOD EPISODES. GO TO MODULE B (PSYCHOTIC AND ASSOCIATED SYMPTOMS), **B1**.

DYSTHYMIC DISORDER CRITERIA

NOTE: For presentations in which there is a history of multiple recurrent Major Depressive Episodes, the clinician may wish to skip the evaluation of Dysthymic Disorder (i.e. go to **B1**).

A45 For the past couple of years, have you been bothered by depressed mood most of the day, more days than not? (More than half of the time?) IF YES: What was that like?	A. Depressed mood for most of the day, for more days than not, as indicated either by subjective account or observation made by others, for at least two years.	**A45** ***?*** **-** ***+***

If A**45** is "-" (ie. No chronic depressed mood. ..) go to **B1** (*Psychotic and Associated Symptoms*)

During these periods of (OWN WORDS FOR CHRONIC DEPRESSION) do you find that most of the time you…	B. Presence, while depressed, of two (or more) of the following:	
A46 . . . lose your appetite? (What about overeating?)	(1) poor appetite or overeating	**A46** **?** **-** **+**
A47 . . . have trouble sleeping or sleep too much?	(2) insomnia or hypersomnia	**A47** **?** **-** **+**
A48 . . . have little energy to do things or feel tired a lot?	(3) low energy or fatigue	**A48** **?** **-** **+**
A49 . . . feel down on yourself? (Feel worthless, or a failure?)	(4) low self-esteem	**A49** **?** **-** **+**
A50 . . . have trouble concentrating or making decisions?	(5) poor concentration or difficulty making decisions	**A50** **?** **-** **+**
A51. . . feel hopeless?	(6) feelings of hopelessness	**A51** **?** **-** **+**
A52 **CODED "+"**	**AT LEAST TWO "B" SYMPTOMS**	**A52** **?** **-** **+**

A53 What is the longest time, during this period of long-lasting depression, that you felt OK? (NO DYSTHYMIC SYMPTOMS)	C. During the 2-year period of the disturbance, the person has never been without the symptoms in criteria A and B for more than 2 months at a time.	**A53** **? -** **+**

If A**53** is "-" (ie. More than 2 months without symptoms) go to **B1** (*Psychotic and Associated*

A54 How long have you been feeling this way? (When did this begin?)	Age at onset of current Dysthymic Disorder (CODE 99 IF UNKNOWN)	**A54**
A55 IF UNKNOWN: Did it begin gradually or did it start with a bad period of depression?	D. No Major Depressive Episode during the first 2 years of the disturbance i.e. not better accounted for by chronic Major Depressive Disorder or Major Depressive Disorder, In Partial Remission. Note: There may have been a previous Major Depressive Episode provided there was a full remission (no significant signs or symptoms for 2 months)before development of the Dysthymic Disorder. In addition, after the initial 2 years of Dysthymic Disorder, there may be superimposed episodes of Major Depressive Disorder, in which case both diagnoses may be given when criteria are met for a Major Depressive Episode.	**A55** **? -** **+**

If A**55** is "-"(i.e. Major Depressive Episode during first 2 years) go to **B1** (*Psychotic and Associated Symptoms*)

A56	E. There has never been a Manic Episode, a Mixed Episode or a Hypomanic Episode, and the criteria have never been met for Cyclothymic Disorder.	**A56** **? -** **+**

If A**56** is "-" (i.e. past Manic, Mixed or Hypomanic Episode or criteria met for Cyclothymic Disorder)

go to **B1** (*Psychotic and Associated Symptoms*)

A57 THIS MAY NEED TO BE DEFERRED UNTIL AFTER PSYCHOTIC DISORDERS HAVE BEEN RULED OUT	F. The disturbance does not occur exclusively during the course of a chronic Psychotic Disorder, such as Schizophrenia or Delusional Disorder.	**A57** **? -** **+**

A58 Just before this began, were you physically ill? Just before this began, were you taking any medications? IF YES: Any change in the amount you were taking? Just before this began, were you drinking or using any street drugs?	G. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or to a general medical condition. <u>Etiological general medical conditions include</u>: degenerative neurological illnesses (e.g., Parkinson's disease), cerebrovascular disease (e.g., stroke), metabolic conditions (e.g., Vitamin B-12 deficiency), endocrine conditions (e.g., hyper- and hypothyroidism, hyper- and hypoadrenocorticism); viral or other infections (e.g., hepatitis, mononucleosis, HIV), and certain cancers (e.g., carcinoma pancreas). gical substances include: alcohol, etamines, cocaine, hallucinogens, nts, opioids, phencyclidine, sedatives, hypnotics, anxiolytics. Medications include antihypertensives, oral contraceptives, corticosteroids, anabolic steroids, anticancer agents, analgesics, anticholinergics, and cardiac medications.	**A58** **? -** **+**

If there is any indication that the dysthymia may be secondary (i.e., a direct physiological consequence of a general medical condition or

If **A58** is "-" (i.e. due to a chronic general medical condition or chronic substance use) go to **B1** (*Psychotic and Associated Symptoms*)

A59 IF UNCLEAR: How much do [SYMPTOMS IN A AND B] interfere with your life?	H. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning	**A59** **? -** **+**

If **A59** is "-" (i.e. not clinically significant) go to **B1** (*Psychotic and Associated Symptoms*)

A60 **CRITERIA A, B, C, D, E, F, G, AND H ARE CODED "+" 300.4 DYSTHYMIC DISORDER**	**A60** **+**

Go to **B1** (*Psychotic and Associated Symptoms*)

CONSIDER ETIOLOGICAL ROLE OF A GENERAL MEDICAL CONDITION OR SUBSTANCE USE

If mood symptoms are <u>not</u> temporarily associated with a general medical condition, go to **A65** *(Substance-induced Mood Disorder)*

MOOD DISORDER DUE TO GENERAL MEDICAL CONDITION CRITERIA

A61 CODE BASED ON INFORMATION ALREADY OBTAINED	A. A prominent and persistent disturbance in mood predominant in the clinical picture and by either (or both) of the following: (1) depressed mood or markedly diminished interest or pleasure in all, or almost all, activities (2) elevated, expansive or irritable mood	**A61** **? -** **+**
A62 Do you think your (MOOD SYMPTOMS) were in any way related to your (COMORBID GMC)? IF YES: Tell me how. (Did the [MOOD SYMPTOMS] start or get much worse only after [COMORBID GMC] began?) IF YES AND GMC HAS RESOLVED: Did the [MOOD SYMPTOMS] get better once the [COMORBID GMC] got better?	B./C. There is evidence from the history, physical examination, or laboratory findings that the disturbance is the direct physiological consequence of a general medical condition and the disturbance is not better accounted for by another mental disorder (e.g. Adjustrment Disorder With Depressed Mood in response to the stress of having a general medical condition).	**A62** **? -** **+**

If A**62** is "-" (GMC not etiological) go to **A65** (*Substance-induced Mood Disorder*)

A63 IF UNCLEAR: How much did (MOOD SYMPTOMS) interfere with your life?	E. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning	**A63** **? -** **+**
A64 CRITERIA A, B/C AND E ARE + **293.83 MOOD DISORDER DUE TO A GENERAL MEDICAL CONDITION** IF UNKNOWN: Have you had (SYMPTOMS RATED "+" ABOVE) in the past month? Check here _____ if criteria have been met in the past month		**A64** **+**

If mood symptoms are not temporarily associated with substance use, return to episode being evaluated:

A12 for Major Depressive Episode **A27** for Manic Episode **A42** for Hypomanic Episode

SUBSTANCE-INDUCED MOOD DISORDER CRITERIA

A65 CODE BASED ON INFORMATION ALREADY OBTAINED	A. A prominent and persistent disturbance in mood predominant in the clinical picture and by either (or both) of the following: 1) depressed mood or markedly diminished interest or pleasure in all, or almost all, activities (2) elevated, expansive or irritable mood	**A65** **? -** **+**
A66 IF UNKNOWN: When did the (MOOD SYMPTOMS) begin? Were you already using (SUBSTANCE) or had you just stopped or cut down your use?	B. There is evidence from the history, physical examination, or laboratory findings of either (1) or (2): (1) the symptoms in criterion A developed during or, within a month of, Substance Intoxication or Withdrawal (2) medication use is etiologically related to the disturbance	**A66** **? -** **+**

If **A66** is "-" (i.e. not etiologically related to a substance), then return to episode being evaluated:

A12 for Major Depressive Episode **A27** for Manic Episode **A42** for Hypomanic Episode

A67 Do you think your (MOOD SYMPTOMS) are in any way related to your (SUBSTANCE USE)? IF YES: Tell me how. ASK ANY OF THE FOLLOWING QUESTIONS AS NEEDED TO RULE OUT A NON SUBSTANCE- ETIOLOGY IF UNKNOWN: Which came first, the SUBSTANCE USE) or the (MOOD SYMPTOMS)? IF UNKNOWN: Have you had a period of time when you stopped using (SUBSTANCE)? IF YES: After you stopped using (SUBSTANCE) did the (MOOD SYMPTOMS) get better? IF UNKNOWN: How much of (SUBSTANCE) were you using when you began to have (MOOD SYMPTOMS)? IF UNKNOWN: Have you had any other episodes of (MOOD SYMPTOMS)? IF YES: How many? Were you using (SUBSTANCES) at those times?	C. The disturbance is not better accounted for by a Mood Disorder that is not substance-induced. Evidence that the symptoms are better accounted for by a Mood Disorder that is not substance induced might include: 1) the mood symptoms precede the onset of the substance use (or medication use) 2) the mood symptoms persist for a substantial period of time (e.g. about a month) after the cessation of acute withdrawal or severe intoxication 3) the mood symptoms are substantially in excess of what would be expected given the type or amount of the substance used or the duration of use 4) there is other evidence that suggests the existence of an independent non-substance-induced Mood Disorder (e.g. a history of recurrent non-substance-related Major Depressive Episodes)	**A67** **? - +**

If **A67** is "-" (i.e. the disturbance is better accounted for by a non-substance-induced Mood Disorder) then return to episode being evaluated:

A68 IF UNKNOWN: How much did (MOOD SYMPTOMS) interfere with your life?	E. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.	**A68** **? -** **+**
A69 CRITERIA A, B/C AND E ARE + **SUBSTANCE-INDUCED MOOD DISORDER** **Code 291.8 for Alcohol, 292.84 for all other substances**. IF UNKNOWN: Have you had (SYMPTOMS RATED "+" ABOVE) in the past month? Check here _____ if criteria have been met in the past month		**A69** **+**

Return to episode being evaluated:		
A12 for Major Depressive Episode	**A27** for Manic Episode	**A42** for Hypomanic

SCID-I DIAGNOSTIC SUMMARY

MOOD DISORDERS

Current Lifetime Bipolar I Disorder

❒ ❒ 296.40 Bipolar I Disorder, Most Recent Episode Hypomanic
❒ ❒ 296.0x Bipolar I Disorder, Single Manic Episode
❒ ❒ 296.4x Bipolar I Disorder, Most Recent Manic Episode
❒ ❒ 296.6x Bipolar I Disorder, Most Recent Episode Mixed
❒ ❒ 296.5x Bipolar I Disorder, Most Recent Episode Depressed

Check fifth digit specifier:

__ 1 - Mild
__ 2 - Moderate
__ 3 - Severe, Without Psychotic Features
__ 4 - Severe, With Psychotic Features
__ 5 - In Partial Remission
__ 6 – In Full Remission
__ 0 – Unspecified

❒ ❒ 296.7 Bipolar I Disorder, Most Recent Episode Unspecified

Other Bipolar Disorders

❒ ❒ 296.89 Bipolar II Disorder (D9)

Check specifier:

__ Hypomanic
__ Depressed

❒ ❒ 301.13 Cyclothymic Disorder (D12)
❒ ❒ 296.80 Bipolar Disorder Not Otherwise Specified (D12)

Major Depressive Disorder (D16)

❒ ❒ 296.2x Major Depressive Disorder, Single Episode
❒ ❒ 296.3x Major Depressive Disorder, Recurrent

Check fifth digit specifier:

__ 1 - Mild
__ 2 - Moderate
__ 3 - Severe, Without Psychotic Features
__ 4 - Severe, With Psychotic Features
__ 5 - In Partial Remission
__ 6 – In Full Remission
__ 0 – Unspecified

Other Depressive Disorders

❒ 300.4 Dysthymic Disorder (A60)
❒ ❒ 311 Depressive Disorder Not Otherwise Specified (D19)

Other Mood Disorders

❒ ❒ 293.83 Mood Disorders Due to General Medical Condition (A64)

Indicate GMC _____

Check specifier:

__ Major Depressive-like Episode
__ Other Depressive Symptoms
__ Manic
__ Mixed

❒ ❒ 291.8 Alcohol-Induced Mood Disorder (A69)

Check specifier:

__ Depressed
__ Manic
__ Mixed

❒ ❒ 292.84 Other Substance-Induced Mood Disorder (A69)

Indicate Substance: _____

Check specifier:

__ Depressed
__ Manic
__ Mixed

Appendix G: Hamilton Rating Scale for Depression (HAM-D)

HAM -D

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1. DEPRESSED MOOD
(*Sadness, hopeless, helpless, worthless*)

0=Absent
1=These feelings are indicated only on questioning
2=These feelings are spontaneously reported verbally
3=Communicates feelings non-verbally i.e., through facial expression, posture, voice, and tendency to weep
4=Patient reports VIRTUALLY ONLY these feelings in his spontaneous verbal and non-verbal communication

2. FEELINGS OF GUILT

0=Absent
1=Self reproach, feels he has let people down
2=Ideas of guilt or rumination over past errors or sinful deed
3=Present illness is a punishmnent. Delusions of guilt
4=Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

3. SUICIDE

0=Absent
1=Feels life is not worth living
2=Wishes he were dead or any thoughts of possible death to self
3=Suicide ideas or gesture
4=Attempts at suicide (any serious attempt rates)

4. INSOMNIA EARLY

0=No difficulty falling asleep
1=Complains of occasional difficulty falling asleep - more than 1/2 hour
2=Complains of nightly difficulty falling asleep

5. INSOMNIA MIDDLE

0=No difficulty
1=Patient complains of being restless and disturbed during the night
2=Waking during the night - any getting out of bed (except for purposes of voiding)

6. INSOMNIA LATE

0=No difficulty
1=Waking in early hours of the morning but goes back to sleep
2=Unable to fall asleep again if he gets out of bed

7. WORK AND ACTIVITIES

0=No difficulty
1=Thoughts and feelings of incapacity, fatigue or weakness related to activities (work or hobbies)
2=Loss of interest in activities (hobbies or work) - either directly reported by patient, or indirectly

in listlessness, indecision and vacillation (feels he has to push himself to work or do activities)
3=Decrease in actual time spent in activities or decrease in productivity. In hospital, if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores
4=Stopped working because of present illness. In hospital, if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted

8. RETARDATION: PSYCHOMOTOR
(Slowness of thought and speech; impaired ability to concentrate; decreased motor activity)

0=Normal speech and thought
1=Slight retardation at interview
2=Obvious retardation at interview
3=Interview difficult
4=Complete stupor

9. AGITATION

0=None
1=Fidgetiness
2=Playing with hands, hair,etc
3=Moving about, can't sit still
4=Hand wringing, nail biting, hair-pulling, biting of lips

10. ANXIETY: PSYCHIC

0=No difficulty
1=Subjective tension and irritability
2=Worrying about minor matters
3=Apprehensive attitude apparent in face or speech
4=Fears expressed without questioning

11. ANXIETY: SOMATIC
(Physiological concomitants of anxiety, such as - Gastro-intestinal: dry mouth, wind, indigestion, diarrhea, cramps, belching. - Cardio-vascular : palpitations, headaches. - Respiratory: hyperventilation, sighing. - Urinary frequency - Sweating)

0=Absent
1=Mild
2=Moderate
3=Severe
4=Incapacitating

12. SOMATIC SYMPTOMS: GASTROINTESTINAL

0=None
1=Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen
2=Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for gastro-intestinal symptoms

13. SOMATIC SYMPTOMS: GENERAL

0=None
1=Heaviness in limbs, back or head. Backaches, headache, muscle aches. Loss of energy and fatigability
2=Any clear-cut symptom

14. GENITAL SYMPTOMS
(loss of libido, menstrual disturbances)

0=Absent
1=Mild
2=Severe

15. HYPOCHONDRIASIS

0=Not present
1=Self-absorption (bodily)
2=Preoccupation with health
3=Frequent complaints, requests for help, etc. ...
4=Hypochondriacal delusions

16. LOSS OF WEIGHT

0=No weight loss
1=Probable weight loss associated with present illness (>500g/week)
2=Definite weight loss(>1kg/week)

17. INSIGHT

0=Not depressed (based on above items) OR Acknowledges being depressed and ill
1=Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2=Denies being ill at all

Appendix H: Childhood Experience of Care and Abuse Questionnaire (CECA.Q)

CECA.Q

1. WHO BROUGHT YOU UP BEFORE AGE 17

Please write below the *Parent Figures* who brought you up in childhood. List each family arrangement with different parent figures which lasted *one year or longer*.
Consider natural parent, step-parent (including parent's live-in partner), aunt, friend of the family, adoptive parent, foster parent etc.

Fill in the first family arrangement below. For example, if this was with your natural parents, write in 'Mother' and 'Father' and age '0'; or if this was with just your mother write in 'Mother', put 'No father figure' in the father column, and age '0'.

Family arrangement	**Mother figure**	**Father figure**	**Your age at start**
FIRST family	1a	1b	1c

If you have lived in *more than just one* family arrangement, such as with mother and stepfather, then list them below, together with the age you were when the arrangement began.

Family arrangement	**Mother figure**	**Father figure**	**Your age at start**
SECOND **family**	1d	1e	1f
THIRD family	1g	1h	1i
FOURTH **family**	1j	1k	1l
FIFTH family	1m	1n	1o

1p ****Were you ever in a children's home or institution before age 17? YES NO**
(please circle the appropriate answer)

If 'YES' fill in the boxes below. If 'NO' skip to question 2 overleaf

TYPE OF INSTITUTION e.g. local authority care, hospital, boarding school	**age when you started**	**age when you left**
1st (1q)	1r	1s

2nd (1t)	1u	1v

2. PARENTAL LOSS

Please circle the appropriate answers, and write in the age you were when it happened.

	MOTHER	**FATHER**
2a. Did either parent die before you were aged 17?	YES NO	YES NO
If YES, what age were you?	2b	2c

	MOTHER	**FATHER**
2d. Have you ever been separated from your parent *for one year or more* before the age of 17?	YES NO	YES NO

If YES, then fill in the boxes below; if NO then SKIP to question 3 overleaf.

	MOTHER	**FATHER**
At what age were you first separated?	2e	2f
How long was this separation, in years?	2g	2h
Please circle the reason for the separation:		
Parent's illness (2i)	YES NO	YES NO
Parent's work (2j)	YES NO	YES NO
Parents' divorce or separation (2k)	YES NO	YES NO
Abandoned by parent or never knew parent (2l)	YES NO	YES NO
Other reason (2m)	YES NO	YES NO

2n. Please describe your experience…….

……..

……

3. Please circle the appropriate numbers to describe your **Mother Figure, as you remember her in your first 17 years**. If you had more than one, choose the one you were with *the longest*, or the one you found *most difficult* to live with.

3a. Which mother figure are you describing below?

1. Natural mother
2. Step-mother/father's live-in partner
3. Other relative e.g aunty, grandmother
4. Other non-relative e.g. foster mother, godmother
5. Other (describe)……

	Yes, definitely		Unsure		No, not at all
3b She was very difficult to please	1	2	3	4	5
3c She was concerned about my worries	1	2	3	4	5
3d She was interested in how I did at school	1	2	3	4	5
3e She made me feel unwanted	1	2	3	4	5
3f She tried to make me feel better when I was upset	1	2	3	4	5
3g She was very critical of me	1	2	3	4	5
3h She would leave me unsupervised before I was 10 years old	1	2	3	4	5
3i She would usually have time to talk to me	1	2	3	4	5
3j She would hit me	1	2	3	4	5
3k At times she made me feel I was a nuisance	1	2	3	4	5
3l She often picked on me unfairly	1	2	3	4	5
3m She was there if I needed her	1	2	3	4	5
3n She was interested in who my friends were	1	2	3	4	5
3o She was concerned about my whereabouts	1	2	3	4	5
3p She cared for me when I was ill	1	2	3	4	5
3q She neglected my basic needs (e.g. food and clothes)	1	2	3	4	5
3r She did not like me as much as my brothers and sisters (*leave blank if no siblings*)	1	2	3	4	5

3s. Do you want to add anything about your mother?……..
……..

4. Please circle the appropriate numbers to describe **your Father Figure, as you remember him in your first 17 years**. If you had more than one, choose the one you were with *the longest*, or the one you found *most difficult* to live with.

4a. Which father figure are you describing below?

1. Natural father
2. Step-father/mother's live-in partner
3. Other relative e.g uncle, grandfather
4. Other non-relative e.g. foster father, adoptive father
5. Other (describe)……

	Yes, definitely		Unsure		No, not at all
4b He was very difficult to please	1	2	3	4	5
4c He was concerned about my worries	1	2	3	4	5
4d He was interested in how I did at school	1	2	3	4	5
4e He made me feel unwanted	1	2	3	4	5
4f He tried to make me feel better when I was upset	1	2	3	4	5
4g He was very critical of me	1	2	3	4	5
4h He would leave me unsupervised before I was 10 years old	1	2	3	4	5
4i He would usually have time to talk to me	1	2	3	4	5
4j He would hit me	1	2	3	4	5
4k At times he made me feel I was a nuisance	1	2	3	4	5
4l He often picked on me unfairly	1	2	3	4	5
4m He was there if I needed him	1	2	3	4	5
4n He was interested in who my friends were	1	2	3	4	5
4o He was concerned about my whereabouts	1	2	3	4	5
4p He cared for me when I was ill	1	2	3	4	5
4q He neglected my basic needs (e.g. food and clothes)	1	2	3	4	5
4r He did not like me as much as my brothers and sisters (*leave blank if no siblings*)	1	2	3	4	5

4s. Do you want to add anything about your father?……

……..

5. CLOSE RELATIONSHIPS IN CHILDHOOD

(please circle as appropriate – if you circle NO to any question, SKIP the rest of that section and go on to the next one)

5a When you were a child or teenager, were there any ADULTS you could go to with your problems or to discuss your feelings?

YES NO

5b If YES: Who was that? (circle more than one if relevant)

1. mother / mother figure
2. father / father figure
3. other relative
4. family friend
5. teacher, vicar etc
6. other (describe) ……..

5d Do you want to note anything about the relationship(s)? ……

5e Were there other CHILDREN/TEENAGERS your age that you could discuss your problems and feelings with?

YES NO

5f If YES: Who was that? (circle more than one if relevant)

1. sister
2. brother
3. other relative
4. close friend
5. other less close friend(s)
6. other person (describe)……..

5h Do you want to note anything about the relationship(s)?……

5i Who would you describe as the TWO CLOSEST people to you as a child/teenager? (circle up to two)

1. mother / mother figure
2. father / father figure
3. sister or brother
4. other relative
5. family friend (adult)
6. friend your age
7. other (describe) …….

5k Do you want to note anything about the relationship(s)?……

6. PHYSICAL PUNISHMENT BEFORE AGE 17 BY PARENT FIGURE OR OTHER HOUSEHOLD MEMBER

6a When you were a child or teenager were you ever hit repeatedly with an implement (such as a belt or stick) or punched, kicked or burnt by someone in the household?

YES NO

If YES, then fill in the boxes below; if NO then SKIP to question 7 overleaf.

	MOTHER FIGURE	**FATHER FIGURE**
How old were you when it began, in years?	6b	6c
Did the hitting happen on more than one occasion?	6d YES NO	YES NO
How were you hit?	6e 1. belt or stick 2. punched/kicked 3. hit with hand 4. other	6f 1. belt or stick 2. punched/kicked 3. hit with hand 4. other
Were you ever injured e.g. bruises, black eyes, broken limbs?	6g YES NO	YES NO
Was this person so angry they seemed out of control?	6h YES NO	YES NO

6i. Can you describe these experiences ……..

…….

…….

6j. Did you experience this from anyone else in the household? YES NO

6k. If YES: describe your experiences…….

…….

…….

7. UNWANTED SEXUAL EXPERIENCES BEFORE AGE 17
(please circle as appropriate)

7a. When you were a child or teenager did you ever have any unwanted sexual experiences? **YES NO UNSURE**

7b. Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17? **YES NO UNSURE**

7c. Can you remember any upsetting sexual experiences before age 17 with a related adult or someone in authority e.g. a teacher? YES NO UNSURE

If NO to all these,FINISH
If YES or UNSURE to any of them, then please complete the following questions:

	FIRST EXPERIENCE	**SECOND EXPERIENCE**
What age were you when it began (in years)?	7d	7l
Was the other person someone you knew?	7e YES NO	7m YES NO
Was the other person a relative?	7f YES NO	7n YES NO
Did the other person live in your household?	7g YES NO	7o YES NO
Did this person do it to you on more than one occasion?	7h YES NO	7p YES NO
Did it involve touching private parts of your body?	7i YES NO	7q YES NO
Did it involve touching private parts of the other person's body?	7j YES NO	7r YES NO
Did it involve sexual intercourse?	7k YES NO	7s YES NO

7t. Can you describe these experiences?……

……

……

Appendix I: 11 and 16 years maltreatment interview

Obtained as part of the Child and Adolescent Psychiatric Assessment (CAPA)

PHYSICAL ABUSE
Subject has been the victim of physical abuse by a member of the family.
Has anyone in your family ever hit or hurt you badly?
Or beaten you up really badly? What happened? Did they threaten you with a weapon? Why did they do it?

Definitions and questions
Coding rules
VICTIM OF PHYSICAL ABUSE: EVER
0 = Absent
2 = Some physical injury (e.g., black eye, cuts), or force with potential for such.
3 = Serious injury (e.g., broken limb, unconsciousness, hospitalization), or force with potential for such.
PERSON USING FORCE: EVER
2 = Parent in the home
3 = Other parent not in the home
4 = Sibling in the home
5 = Sibling not in the home
6 = Other adult family member.
THREATENED WITH WEAPON: EVER
0 = Absent
2 = Weapon used to threaten but not to hurt victim.
3 = Weapon used to threaten and injure victim.

SEXUAL ABUSE OR RAPE
Sexual abuse episode(s) in which a person, termed a perpetrator, involves a child or adolescent in activities for the purpose of the perpetrator's own sexual gratification. These activities can include kissing (that makes a person uncomfortable), genital fondling (over or under clothing), oral-genital or oral-anal contact, genital or anal intercourse, or use of instruments. Sexual abuse does not include medical exams or mutually desires sexual relations with a peer.
Rape is a sudden unexpected (usually isolated) event involving non-consensual sexual intercourse.

Has anyone ever touched you in places where they shouldn't?
Has anyone ever touched you in ways that made you feel funny?
Or seemed wrong to you?
Has anyone ever made you touch them in ways that made you feel uncomfortable?
What happened? Who was involved? How did you feel about it? Were you upset? When did it first happen? How many times has it happened? Has it happened in the last 3 months?

Definitions and questions
Coding rules

SEXUAL ABUSE: EVER
0 = Absent
2 = Present
NUMBER OF TIMES SEXUAL ABUSE: EVER
ONSET SEXUAL ABUSE:
RAPE: EVER
2 = Perpetrator is a stranger
3 = Perpetrator is known individual
ONSET RAPE: EVER
FREQUENCY RAPE: EVER

Appendix J: Protocol for the calculation of high sensitivity C-reactive protein

High sensitivity c reactive protein (hsCRP)

EXPLANATION OF THE TEST

CRP is a 125,000 Dalton tetrameric protein synthesized in the liver that circulates in the plasma at an average concentration of 2.5 mg/L. Its production is regulated by interleukin-6. CRP binds polysaccharides, phosphorylcholine, phosphatidylcholine, histories, and nucleic acids. Once it forms a complex with one of these molecules, CRP activates the classical complement pathway.

METHOD

HsCRP reagents are supplied by P.Z. Cormay, ul. Rapackiego 19, 20-150 Lublin, Poland, P.O. Box 122 20-954 Lublin 2.

The Cormay hsCRP assay employs an anti-CRP antibody that has been sensitized to latex particles. The antibody coats the surface of latex particles forming a milky appearing sensitised latex. This antibody then reacts with CRP within the sample and results in visible agglutination. Latex particles are used to magnify the antigen-antibody complex. The degree of agglutination is detected as a decrease in the intensity of transmitted light at 572nm (turbidimetry), which is proportional to the amount of CRP within the sample. The concentration of CRP is determined by interpolation from a calibration curve prepared from calibrators of known concentration. The assay is analysed on the Siemens Advia 2400.

TECHNICAL DATA

Intra-assay precision

	Level 1	Level 2	Level 3
N	21	21	21
Mean (mg/dL)	0.046	0.228	0.981
SD	0.003	0.005	0.011
CV (%)	5.74	1.99	1.16

Inter-assay Precision

	Level 1	Level 2	Level 3
N	21	21	21
Mean (mg/dL)	0.047	0.218	0.976
SD	0.003	0.007	0.012
CV (%)	6.97	3.34	1.23

Sample requirements

The specimen should be serum from a plain or gel separator vacutainer The usual precautions for venipuncture should be observed.

Serum is aliquoted into a 2mL tube and placed in the fridge for up to 4 hours. For longer storage samples should be stored in the freezer at -20°C or below until assayed.

Avoid repeat freeze-thaw cycles. Avoid using samples with gross haemolysis or lipaemia.

To assay the sample in duplicate a minimum of 200 µL is required. However, the sample tube should contain at least a volume of 500 µL to account for the dead volume and repeats.

Sensitivity

The minimum detectable concentration of hsCRP is 0.01 mg/dL.

Reference range

Low risk: less than 1.0 mg/L
Average risk: 1.0 to 3.0 mg/L
High risk: above 3.0 mg/L

Women on hormone replacement therapy have been shown to have elevated hs-CRP levels, suggesting that this test may be useful in predicting future cardiovascular events.

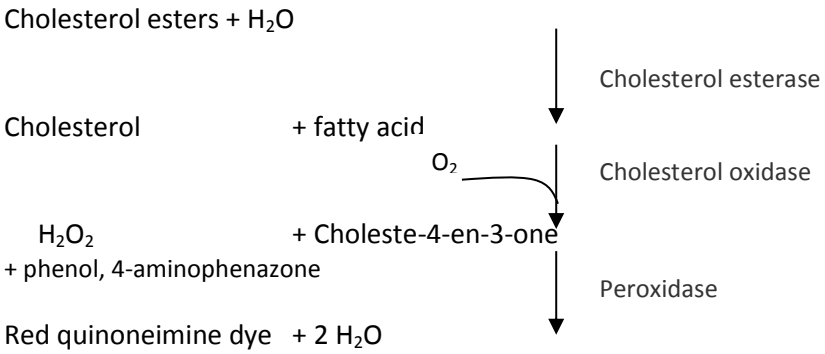
Appendix K: Protocols for the calculations of metabolic parameters

CHOLESTEROL

METHOD

Cholesterol reagent supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, Gu16 8QD

Serum cholesterol is determined using an enzymatic method. Cholesterol esterase completely hydrolyses cholesterol esters in serum to free cholesterol, which is in turn oxidised by cholesterol oxidase generating hydrogen peroxide. The hydrogen peroxide formed combines with 4-aminophenazone and a phenol to form a red quinone amine dye which is measured as an endpoint reaction at 505/694 nm. The increase in dye absorbance is directly proportional to the concentration of cholesterol in the sample, when compared to a previous calibration assay.



TECHNICAL DATA

Intra-assay precision

	Level 1)	Level 2	Level 3
N	20	20	20
Mean (mmol/L)	3.91	5.15	5.67
SD	0.026	0.034	0.031
CV%	0.6	0.6	0.6

Inter-assay precision

	Level 1	Level 2	Level 3
N	20	20	20
Mean (mmol/L)	3.91	5.15	5.67
SD	0.044	0.080	0.057
CV%	1.1	1.5	1.0

Sample requirements

Serum or heparinised plasma may be used. Specimens may be refrigerated overnight or stored at room temperature in seroseparator tubes. The ADVIA 2400 uses 2 µl of sample in the test, although the minimum volume required in the specimen container is 50µl (micro sample cups) or 200µl (Vacutainers and inserts).

Linearity

Linear to approx. 17 mmol/l.

Sensitivity

The minimum detectable concentration of cholesterol is 0.01 mmol/L.

Reference range

Quoting reference values for serum cholesterol in terms of population distribution is actively discouraged in favour of setting desirable targets. Current WHO recommendations suggest a cholesterol concentration below 5.2 mmol/L as being desirable to avoid coronary artery disease.

Standardization

The ADVIA cholesterol method is traceable to the CDC reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation.

TRIGLYCERIDES

Triglyceride concentrations are useful in the calculation of LDL cholesterol via the Friedwald formula given the total and HDL cholesterol.

Method

Triglyceride reagents supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, Gu16 8QD

The Siemens Advia method for the measurement of triglycerides is an enzymatic assay. Triglycerides are converted to glycerol and free fatty acids by lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion by glycerol-3-phosphate-oxidase to hydrogen peroxide. A coloured complex is formed from hydrogen

peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.

Technical

Intra-assay precision

	Level 1	Level 2
Mean (mmol/L)	1.32	2.36
N	20	20
SD	0.007	0.011
CV%	0.6	0.5

Inter-assay precision

	Level 1	Level 2
Mean (mmol/L)	1.32	2.36
N	20	20
SD	0.033	0.035
CV%	2.5	1.5

Sample requirements

Serum or heparinised plasma may be used. Specimens may be refrigerated overnight or stored at room temperature in seroseparator tubes. The ADVIA 2400 uses 2 µL of sample in the test, although the minimum volume required in the specimen container is 50µL (micro sample cups) or 200µL (Vacutainers and inserts).

Linearity

Linear to approximately 10 mmol/L.

Sensitivity

The minimum detectable concentration of triglyceride is 0.01 mmol/L.

Standardization

The ADVIA triglyceride method measures total glycerols and is traceable to a reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation. Assigned values of Bayer Chemistry Calibrator and Bayer Assayed Chemistry Controls are traceable to this standardization.

Reference Range

< 2.0 mmol/L (fasting).

HIGH DENSITY LIPOPROTEIN CHOL

HDL cholesterol measurements enable LDL cholesterol to be calculated (given the total cholesterol and triglycerides)

Method

HDL cholesterol reagents supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, Gu16 8QD.

The Siemens Advia Direct HDL cholesterol method is a two step automated procedure. In the first step cholesterol esterase and cholesterol oxidase react to remove non-HDL cholesterol from the sample. The hydrogen peroxide produced is then removed by the enzyme catalase. The absence of detergent in this first reaction prevents HDL from reacting with the enzymes. In stage 2 detergent is added to allow HDL to react with the enzyme system. Sodium azide inhibits the reaction of the hydrogen peroxide formed with catalase. The hydrogen peroxide acts with 4-aninoantipyrine to produce a quinoneimine pigment measured at 596 nm.

Technical

Intra-assay precision

	Level 1	Level 2	Level 3
Mean (mmol/L)	0.91	1.39	1.95
N	20	20	20
SD	0.010	0.019	0.029
CV%	1.1	1.4	1.5

Inter-assay precision

	Level 1	Level 2	Level 3
Mean (mmol/L)	0.91	1.39	1.95
N	20	20	20
SD	0.020	0.029	0.049
CV%	2.2	2.1	2.5

Sample requirements

Serum or heparinised plasma may be used. Specimens may be refrigerated overnight or stored at room temperature in seroseparator tubes. The ADVIA 2400 uses 2 µl of sample in the test, although the minimum volume required in the specimen container is 50µl (micro sample cups) or 200µl (Vacutainers and inserts).

Sensitivity

The minimum detectable concentration of HDL cholesterol is 0.1 mmol/L.

Linearity

Linear from 0.4 - 2.3 mmol/L.

Reference Range

Quoting reference values for serum HDL cholesterol in terms of population distribution is actively discouraged in favour of setting desirable targets. Current WHO recommendations suggest a cholesterol concentration above 1.0 mmol/l as being desirable to avoid coronary artery disease. HDL cholesterol is also used in conjunction with total cholesterol and triglycerides to calcualate LDL cholesterol.

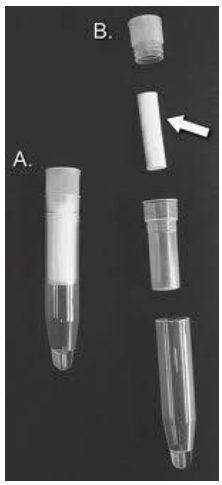
Standardization

The Advia HDL method is traceable to the NCEP Designated Comparison Method (reference method) via patient sample correlation. Assigned values of ADVIA Chemistry HDL/LDL Cholesterol Calibrator and ADVIA Chemistry Lipid Controls are traceable to this standardization.

Appendix L: Saliva sampling instructions and record log

How to collect the saliva samples:

Salivette with swab

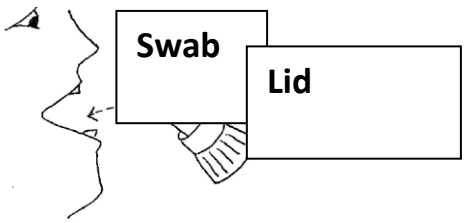


Lid
Swab
Inner tube
Outer tube

1. **Take care to find the salivette tube marked with the appropriate time.**

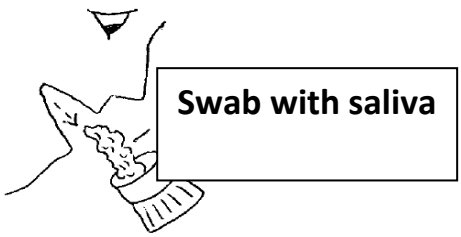
2. **Carefully remove the lid (the part on the end with ridges on).**

3. **Tip the swab into the lid and use this to place the swab under the front of your tongue. Do not touch the swab with your fingers.**



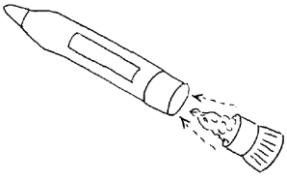
4. **Keep the swab in place for 1-2 minutes to ensure that it is saturated.**

5. **Take the swab out of your mouth with the help of the lid (so you are not touching the swab with your fingers).**



6. **Carefully tip the swab into the salivette tube without touching it with your fingers.**

7. **Replace the lid firmly.**



8. **Store the samples in your fridge.**

THANK YOU.

South London Child Development Study
SALIVA COLLECTION RECORD

PLEASE TAKE THE SAMPLES ON THE WEEKEND

Date of collection *dd/mm/yy*	……/……../……	**ID**				

PLEASE ENSURE YOU TAKE YOUR SAMPLES AT THE CORRECT TIMES		
	Time	**Complete**
Awakening		BOX 1
Awakening +15 minutes		BOX 2
Awakening +30 minutes		BOX 3
Awakening +60 minutes		BOX 4
12pm		BOX 5
8pm		BOX 6

BOX 1

Wake up (before 10am)
Immediately after waking up collect your saliva as on the instruction diagram. Place the swab ***under your tongue*** and leave it there for 1-2 minutes, then place it as shown in the **tube marked awakening**.
Then close the tube firmly and store in the fridge in the bag supplied.

Write here the **EXACT TIME OF AWAKENING:** _____

PLEASE NOW USE THE TIMER PROVIDED
Try to sit down and relax in the next hour. **YOU MUST NOT BRUSH YOUR TEETH AND MUST NOT HAVE ANYTHING TO EAT, DRINK OR SMOKE FOR THE NEXT HOUR**.
You may drink water if you need to, but only immediately AFTER you have taken a sample.

BOX 2

15 minutes after waking up, collect your saliva in the **tube marked 15 mins**.
Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now?
  _____
- What were you doing before giving the sample?
  _____
  _____
- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:
  _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:
  _____
  _____

BOX 3

30 minutes after waking up, collect your saliva in the **tube marked 30 mins**.

Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

  _____

_____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

  _____

  ____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

  _____

_____

BOX 4

60 minutes after waking up, collect your saliva in the **tube marked 60 mins**.
Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

  _____

_____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

  _____

  ___

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

  _____

_____

*******YOU CAN NOW HAVE BREAKFAST AND BRUSH YOUR TEETH! *******
*******YOU SHOULD NOT EAT OR DRINK ANYTHING, SMOKE OR BRUSH YOU TEETH IN THE 30 MINUTES BEFORE NOON.*******

BOX 5

At 12 noon, before lunch, collect your saliva in the **tube marked 12 pm**.
Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now?

  _____

- What were you doing before giving the sample?

  _____

  _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

  _____

- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

_____

_____

*******<u>YOU SHOULD NOT EAT OR DRINK ANYTHING, SMOKE OR BRUSH YOUR TEETH IN THE 30 MINUTES BEFORE 8PM</u>.*******

<u>BOX 6</u>

At 8pm collect your saliva in the **tube marked 8 pm**.
Then close the tube firmly and store in the fridge in the bag supplied

- What time is it now?

  _____
- What were you doing before giving the sample?

  _____

  _____
- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

  _____

  ___
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:

  _____

  _____

Please note the name and time of **any medication you have taken <u>today</u>** (including the contraceptive pill):
_____

Were you ill today, please state if you were and symptoms (i.e. nausea, diarrhoea)
_____

Posting the samples:
Please post your samples back to us AS SOON AS POSSIBLE, **keeping your sample in the fridge prior to posting.**

Place all the samples in the plastic bag provided and seal it carefully. Place the bag and the collection record into the stamped addressed envelope provided and **deposit at the post office**.

_____

Office use only:
[Date of sample receipt: __ / __ / __ Date of sample storage: __ / __ / __]

Appendix M: Assay procedure for salivary cortisol

Saliva was collected using Sarstdet swabs and tubes containing samples were stored at -20C. Immediately before analysis samples were thawed and collection tubes were subjected to 15 min of centrifugation at 3000 rpm. Determination of cortisol levels was done using the High Sensitivity Salivary Cortisol ELISA KIT from Salimetrics, following the recommended procedure.

Briefly, 25 ul of saliva and standards were assayed in duplicates, by incubation on a microtitre plate coated with monoclonal antibodies against cortisol. Cortisol linked to horseradish peroxidase was added to compete with cortisol in the standards and unknowns for the antibody binding sites. After 1h of incubation, unbound components were washed away. Bound cortisol peroxidase was measured by reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. The amount of cortisol peroxidase detected, as measured by the intensity of colour developed, was inversely proportional to the amount of cortisol present. Optical density was read at 450 nm with correction at 620 nm, using a Beckman Coulter DTX 880 plate reader, with Multimode Detection Software 2.0.0.12. Values of cortisol were calculated using SoftMax Pro 4.8 software, following a 4-parameter fit.

All samples from the same participant were analysed in the same plate.

Sensitivity: we set it to 0.33 nmol/l, equivalent to 0.012ug/dl (more stringent than the suggested 0.003 ug/dl)

Inter assay: level 1 8%, level 2 11% (our values)
Intra assay: level 1 5.6% level 2 9.6% (our values)